

2011

The effect of soy protein beverages on serum cell adhesion molecule concentrations in prehypertensive/stage 1 hypertensive individuals

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The effect of soy protein beverages on serum cell adhesion molecule concentrations in prehypertensive/stage 1 hypertensive individuals

by

Michelle Elise Dettmer

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of
MASTERS OF SCIENCE

Major: Diet and Exercise

Program of Study Committee:
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Ames, Iowa

2011

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LIST OF ABBREVIATIONS

Ang II	Angiotensin II
CAM	Cell adhesion molecules
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
E-selectin	Endothelial-leukocyte adhesion molecule
HDL	High-density lipoprotein
HTN	Hypertension
ICAM-1	Intercellular adhesion molecule-1
LDL	Low-density lipoprotein
SBP	Systolic blood pressure
SPI	Soy protein isolate
VCAM-1	Vascular cell adhesion molecule-1
WSB	Whole soy bean

ABSTRACT

Background and Aims: Prehypertensive and hypertensive individuals are at an increased risk of atherosclerotic cardiovascular disease. The role of hypertension in endothelial dysfunction and increased cell adhesion molecule (CAM) expression may lead to atherosclerotic progression. Soy protein and isoflavones have been shown to favorably alter cardiovascular disease risk factors. The aim of this study was to determine the effect of daily cow's milk compared with soy beverage prepared from whole soy bean (WSB) or soy protein isolate (SPI) on soluble cell adhesion molecules.

Methods: We enrolled healthy prehypertensive/Stage 1 hypertensive men (n=60, aged 18 – 63 yr) and premenopausal women (n=8, aged 20 – 48 yr) and randomized them to one of three beverage groups for 8 weeks of treatment: cow's milk (600 mL/d), soy beverage (840 mL/d) prepared from SPI (30.1 mg total isoflavones/d [aglycone form]) or prepared from WSB (91.4 mg total isoflavones/d [aglycone form]). We measured soluble vascular cell adhesion molecule-1 (VCAM-1), intercellular cell adhesion molecule-1 (ICAM-1), and endothelial-leukocyte adhesion molecule-1 (E-selectin) at baseline and week 8.

Results: Treatment did not alter soluble CAM concentrations. Time had an effect on VCAM-1 (-9%, $P = 0.01$) and E-selectin (+4%, $P = 0.01$) but not ICAM-1 (+5%, $P=0.86$). Gender also had a significant effect on ICAM-1 ($P=0.0037$), whereas gender did not reach significance for E-selectin ($P=0.067$) or VCAM-1 ($P=0.16$). Men had higher circulating concentrations of ICAM-1 and E-selectin, respectively, at both baseline ($P = 0.0071$, $P = 0.049$) and week 8 ($P = 0.0054$, $P = 0.038$) than women. ICAM concentrations were not significantly different between prehypertensive and hypertensive participants.

Conclusion: Prehypertensive/Stage I hypertensive individuals who consumed either cow's milk or soy beverages (prepared from WSB or SPI) for 8 weeks daily did not show any change in soluble CAM concentrations. Consequently, we cannot suggest that daily intake of either cow's milk or soy protein beverages improves circulating CAM concentrations and hence risk of atherosclerotic CVD in these individuals.

KEY WORDS: Cardiovascular disease risk, Cell adhesion molecules, VCAM-1, ICAM-1, E-selectin, Isoflavones, Soy protein

GENERAL INTRODUCTION

Thesis Organization

The general introduction of this thesis describes the objectives, hypotheses, specific aims, limitations, and significance of this study. **Figure 1** portrays the overall conceptual model of this study. Pertinent background information follows in the review of literature.

Objectives

The long term objective of this research was to identify a clinical intervention for reducing subclinical inflammation, thereby decreasing risk of atherosclerotic cardiovascular disease (CVD). The primary objective of this study was to determine whether daily intake of soy protein beverage compared with cow's milk, for eight weeks affected concentrations of inflammatory markers in prehypertensive/Stage I hypertensive individuals. The specific inflammatory markers of interest were cell adhesion molecules (CAM): soluble vascular cell adhesion molecule-1 (sVCAM-1), soluble intercellular adhesion molecule-1 (sICAM-1), and soluble endothelial-leukocyte adhesion molecule-1 (sE-selectin). The secondary objective was to observe and compare the magnitude of change in CAM concentrations in response to each treatment and to determine whether gender differences were important.

Hypotheses

1. Daily intake of soy beverages (prepared from either whole soy bean [WSB] or soy protein isolate [SPI]) and cow's milk for eight weeks will reduce inflammation, as evidenced by a decrease in serum sVCAM-1, sICAM-1, and/or sE-selectin, in prehypertensive/Stage I hypertensive individuals.
2. The reduction of serum sVCAM-1, sICAM-1, and sE-selectin concentrations will be greater for individuals who consumed daily a soy protein beverage (prepared from either WSB or SPI) compared to those who consumed cow's milk.
3. Individuals who consumed the soy protein beverage prepared from WSB will experience a greater reduction of serum sVCAM-1, sICAM-1, and sE-selectin compared to individuals who consumed the soy protein beverage prepared from SPI.

Specific Aims

1. To determine change in VCAM-1, ICAM-1, and E-selectin markers of inflammation, as a function of eight weeks of soy beverage consumption compared with cow's milk in prehypertensive/Stage 1 hypertensive individuals.

- a. Serum sVCAM-1, sICAM-1, and sE-selectin were measured via enzyme-linked immunosorbent assay (ELISA)
2. To determine whether soy protein beverage (prepared from either WSB or SPI) reduced sVCAM-1, sICAM-1, sE-selectin concentrations to a greater extent than that of cow's milk.
3. To determine whether soy protein beverage prepared from WSB, as a function of its higher isoflavone content, reduced sVCAM-1, sICAM-1, sE-selectin concentrations to a greater extent than that of soy protein beverage prepared from SPI.
 - a. Concentrations of isoflavones in the soy protein beverages were analyzed by HPLC (Beckman Coulter, Fullerton CA) at Iowa State University (Murphy et al. 1981).

Limitations

The purpose of this study was to determine whether CAM concentrations (indicative of inflammation), specifically VCAM-1, ICAM-1, and E-selectin, would be reduced in response to the intake of soy protein beverages (prepared from either WSB or SPI) compared to cow's milk in prehypertensive/Stage I hypertensive individuals. The inclusion of men and premenopausal women in this study differed from previous research, in that most studies prior to this investigation included postmenopausal women exclusively. Despite our efforts to recruit men and premenopausal women, we did not include both sexes equally (60 men versus 8 women) because premenopausal women with hypertension (HTN) were very difficult to enroll, since they are relatively unusual.

This investigation did not include a true placebo control group, although we included the cow's milk group as a realistic control for the soy beverages. In essence, we were not able to determine the response to a true placebo control in prehypertensive/Stage I hypertensive individuals. However, we did not expect the same magnitude of response from the cow's milk group. Consequently, we will discuss differences in CAM among the three treatment groups post-intervention and changes in CAM within each treatment group from baseline to post-intervention.

This study was designed to examine changes in CAM as biomarkers of inflammation in an at-risk group: prehypertensive/Stage I hypertensive individuals. However, it would be meaningful to compare CAM concentrations between normotensive and prehypertensive/Stage I hypertensive individuals. The inclusion of healthy, normotensive individuals would have provided a comparison of CAM values indicative of minimal inflammation. Because the funding agency specifically did not want us to include a group of normotensive individuals, this has somewhat limited the interpretation of our results. In this study, the statistical analyses focused on the differences in CAM among the three groups post-

treatment, as well as the relative change in CAM from baseline to post-treatment within each group. In addition, previous studies examined primarily postmenopausal women, thereby limiting the availability of a reference range of CAM concentrations from healthy premenopausal women, a target demographic in our study.

Significance and Strengths

Hypertension is a leading risk factor for stroke, coronary heart disease, congestive heart failure (Kannel et al. 1969), peripheral vascular disease, and renal insufficiency (Burt et al. 1995; Chobanian et al. 2003; Stamler et al. 1993; Wang et al. 2005). In the US, 41% of non-Hispanic blacks and 22% of non-Hispanic whites have HTN, many of whom are unaware of their condition (Ostchega et al. 2008). Hypertension is a major risk factor contributing to the pathogenesis of atherosclerotic CVD, the primary cause of death in the US, which is readily preventable and/or controllable (Lloyd-Jones et al. 2010).

Uncomplicated HTN in men compared with normotensive men has been reported to result in elevated VCAM-1 and ICAM-1 concentrations (Chae et al. 2001; DeSousa et al. 1997). Although hypertensive patients may have elevated concentrations of VCAM-1, ICAM-1, and E-selectin compared to normotensive patients, these markers are significantly increased in both groups following an acute rise in blood pressure via a cold pressor test (Buemi et al. 1997). These findings demonstrate a potential role of essential and acute HTN in inflammation and endothelial cell function. However, elevated CAM has not been demonstrated consistently in prehypertensive individuals.

The isoflavones, genistein, daidzein, and glycitein (and their respective glycosides), found in soy foods have anti-inflammatory properties (Chacko et al. 2005; Wei et al. 1995), but this has not been confirmed in uncomplicated HTN (prehypertension/Stage I HTN). Genistein has been shown to have a potent anti-inflammatory action via inhibiting monocyte adhesion to cytokine-activated endothelial cells (Chako et al. 2005). Isoflavones may also have cardioprotective properties similar to endogenous estrogen (Hall et al. 2005). What is not known is whether soy protein intake in prehypertensive/Stage I hypertensive men and premenopausal women not only may decrease blood pressure, but reduce VCAM-1, ICAM-1, and E-selectin concentrations to a greater extent than cow's milk. Should we determine that soy consumption reduces VCAM-1, ICAM-1, and E-selectin in prehypertensive/Stage I hypertensive men and premenopausal women, this would provide evidence that this dietary intervention might decrease inflammation in individuals prior to manifestation of CVD, thereby reducing HTN-related risk of atherosclerotic CVD.

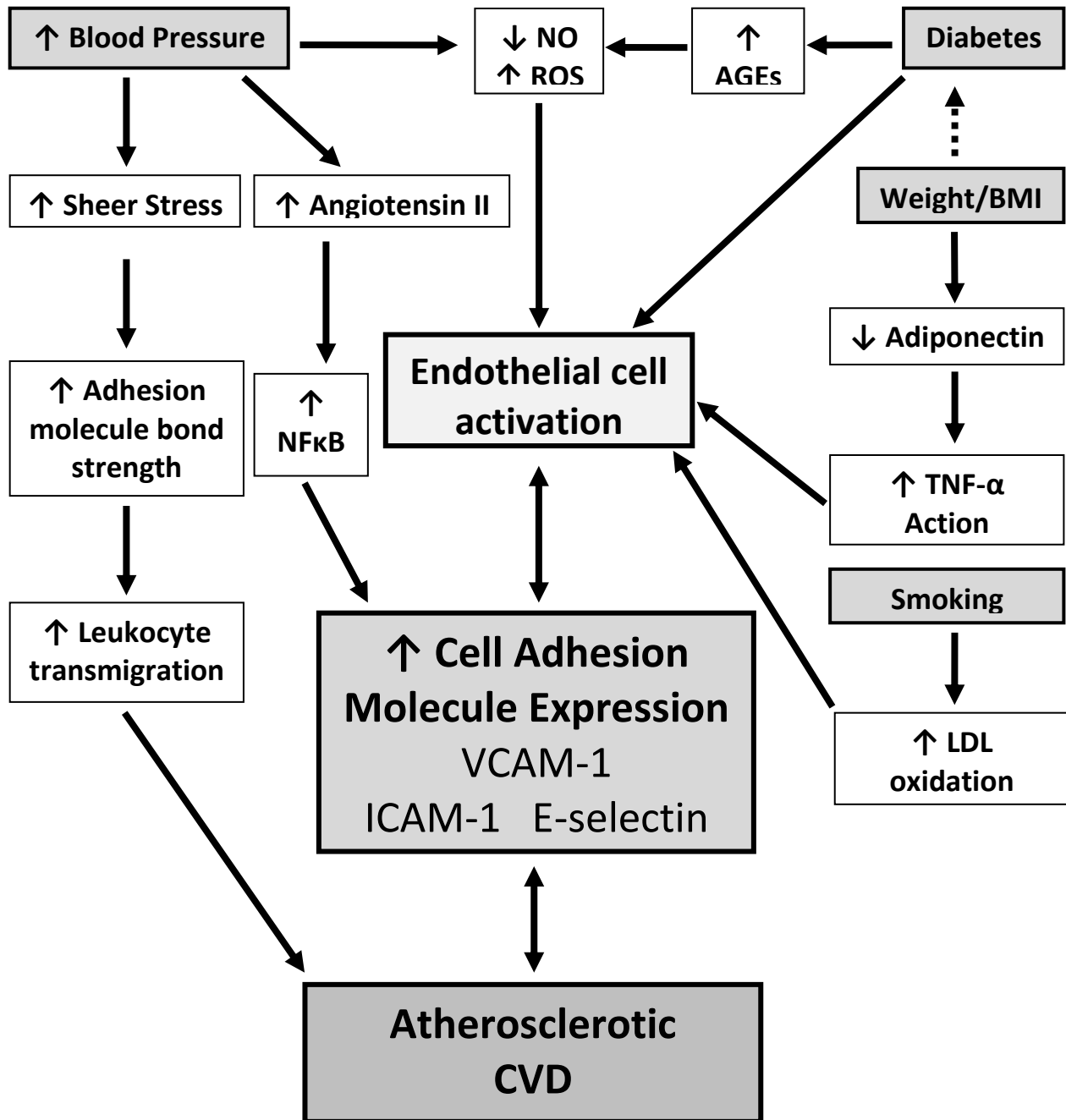


Figure 1. Conceptual Model: Factors related to increased expression of cell adhesion molecules and atherosclerotic CVD. NFκB, nuclear factor kappa B; NO, nitric oxide; ROS, reactive oxygen species; AGE, advanced glycation endproducts; BMI, body mass index; TNF-α, tumor necrosis factor α; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; E-selectin, endothelial-leukocyte adhesion molecule-1; CVD, cardiovascular disease

REVIEW OF LITERATURE

Relationship of Hypertension to Cardiovascular Disease

Hypertension (HTN) is a modifiable risk factor for CVD. Cardiovascular disease is the leading cause of death in the United States, accounting for over 50 percent of all deaths (Lloyd-Jones et al. 2010). Hypertension is defined as a systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg. Normal blood pressure is defined as a SBP < 120 and DBP < 80 . Prehypertension is defined as a SBP 120-139 mmHg and/or a DBP 80-89 mmHg. The Framingham Heart Study has indicated that risk of CVD is more than doubled for individuals with SBP levels 130 to 139 and/or DBP 85 to 89 mmHg compared to individuals with blood pressure levels less than 120/80 mmHg (Vasan et al. 2001). Observational studies that included more than 1 million people, ages 40 to 89, have associated increased death from ischemic heart disease and stroke with either elevated SBP levels ≥ 115 mmHg and/or DBP levels ≥ 75 mmHg (Lewinton et al. 2002). It is estimated that for every 20 mmHg increase in SBP or 10 mmHg increase in DBP, mortality from ischemic heart disease and stroke is doubled (Chobanian et al. 2003).

Although HTN is a well recognized risk factor for CVD, the underlying mechanism related to the pathology of CVD is unknown. Hypertension may be a stimulus for inflammation (Kranzhöfer et al. 1999; Chae et al. 2001) and endothelial activation (Liu et al. 1996), implicating its role in atherogenesis (Ross 1999). Increased blood pressure has been associated with significant increases in the concentration of C-reactive protein (CRP) and interleukin-6 (IL-6), both markers of inflammation (Bermudez et al. 2002; Blake et al. 2003; Chae et al. 2001; Fernandez-Real et al. 2001).

Angiotensin (Ang) II is a primary effector of the renin-angiotensin aldosterone system and a potent vasoconstrictor, playing a crucial role in the regulation of blood pressure (Ross 1999). In hypertensive patients, Ang II is often elevated. Elevated Ang II may contribute to the intimal inflammation evident in hypertensive individuals, as Ang II can stimulate IL-6 expression by smooth muscles cells (Kranzhöfer et al. 1999; Libby et al. 2002). Angiotensin II is also involved in the remodeling of the arterial wall (Matsusaka and Ichikawa 1997) and is known to stimulate oxidative stress in several cell types, including endothelial cells found on the interior surface of blood vessels (Pueyo et al. 1998). Oxidative stress contributes to endothelial damage via the inflammatory cells, polymorphonuclear leukocytes, which release reactive oxygen species (ROS) when activated (Smedly et al. 1986). Hypertensive individuals have been shown to have significantly increased polymorphonuclear leukocyte-induced oxidative stress (Yasunari et al. 2002). The increase in oxidative stress in hypertensive individuals perpetuates the endothelial damage considered to be the first step in atherogenesis.

Risk Factors for Hypertension

Hypertension afflicts an estimated 29 percent of adults in the United States, in addition to 37 percent who have prehypertension (Ostchega et al. 2008). Untreated HTN increases the risk of heart attack, heart failure (Kannel et al. 1969), stroke, and kidney disease (Burt et al. 1995; Chobanian et al. 2003; Stamler et al. 1993; Wang et al. 2005). The risk for these conditions may also be increased in individuals with prehypertension (Vasan et al. 2001), although the evidence is not as clear. The prevalence and grave consequences of this condition are alarming. Although HTN is related to an increased risk of CVD, HTN may be averted. Recent investigations have identified non-modifiable and modifiable risk factors for HTN as summarized herein.

Non-modifiable

Non-modifiable risk factors for HTN include age, gender, race, and genetics. In most populations SBP has been shown to rise disproportionately with advancing age compared to DBP. Both pressures increase with age into adulthood, yet DBP tends to peak in the sixth decade and then decline, while SBP continues to rise into elderly adulthood (Kannel and Gordon 1977). This disproportionate rise in SBP compared to DPB has been related to a progressive loss of arterial elasticity during the lifespan (Kannel and Gordon 1977). In women, longitudinal data suggest that DBP is parallel but persistently lower than men. However, SBP is initially lower in women and eventually converges with that of men near the age of 60. Gender differences in peripheral SBP have also been attributed to differences in body height. Systolic blood pressure amplification from the central to peripheral vasculature increases with height. Because women tend to be shorter than men, this amplification is less pronounced and peripheral SBP is lower in women (London et al. 1995).

Differences in ethnicity have a pronounced impact on blood pressure. The US Department of Health and Human Services that conducts the National Health and Nutrition Examination Survey (NHANES) found that the non-Hispanic black population had the highest prevalence of HTN (41%), significantly higher than those of non-Hispanic white (28%) or Mexican-American (22%) populations (Ostchega et al. 2008). Non-Hispanic blacks have much higher mean blood pressure values and develop high blood pressure earlier in life compared to non-Hispanic whites (Lloyd-Jones et al. 2010). These differences in blood pressure have been attributed to heredity, the reduced ability to photosynthesize vitamin D₃ based on skin melanin content (Rostand 1997), and psychosocial stressors (Kriger and Sidney 1996). High blood pressure may be more prevalent in adult non-Hispanic black populations due to a

higher incidence of obesity (32%) compared to non-Hispanic white (24%) and Asian (9%) adult populations (Pleis and Lethbridge-Cejku 2006).

Genetic regulation of blood pressure occurs in at least eight genome loci (Cheh et al. 2009) that may regulate blood pressure via variations in plasma angiotensinogen concentration (Kim et al. 1994) and renal sodium reabsorption (Lifton et al. 2001), among other yet identified mechanisms. The Framingham Heart Study examined related individuals and found that if measured once, blood pressure levels are almost 40% heritable, and when measured multiple times, blood pressure trends are nearly 55% heritable (Levy et al. 2000).

Modifiable

Modifiable risk factors include excess body weight, physical inactivity, excess sodium intake, excess alcohol intake, diet, and caffeine consumption. Excess body weight and body mass index (BMI; kg/m^2) are positively associated with blood pressure (Dyer et al. 1989). Screening of more than 1 million people revealed self-reported overweight individuals had HTN prevalence rates 50 to 300 percent higher than other individuals for each age-, sex-, and race-group (Stamler et al. 1978). The INTERSALT study associated a 10 kg difference in body weight with an average of 3.0 mmHg difference in SBP and 2.2 mmHg difference in DBP (Dyer et al. 1989). Because of the strong relationship between body weight and blood pressure, weight loss is the primary objective in the prevention and treatment of HTN. It must be noted that although significant relationships between blood pressure and weight have been reported, these values fall within potential measurement error when assessing blood pressure. Thus, it is unclear whether or not these small differences are biologically important. Nevertheless, weight loss has been shown to reduce SBP and DBP in overweight hypertensive patients (Reisin et al. 1983), as well as in overweight patients with prehypertension (Stamler et al. 1989). A meta-analysis that included 25 trials reported that an average weight loss of 5.1 kg reduced SBP by 4.4 mmHg and DBP by 3.5 mmHg (Neter et al. 2003). Furthermore, greater weight loss is associated with greater blood pressure reduction (Huang et al. 1998; Stevens et al. 1993).

Physical fitness is inversely related to SBP (Cooper et al. 1976). Clinical trials have demonstrated a reduction in blood pressure in hypertensive and normotensive subjects with physical activity, independent of weight loss (Arrol and Beaglehole 1992; Whelton et al. 2002). Whelton et al. (2002) conducted a meta-analysis with 54 randomized, controlled trials and concluded that aerobic exercise, performed at least 30 minutes most days of the week, significantly reduced mean SBP by 3.8 mmHg and

mean DBP by 2.6 mmHg. Because these declines approximate the measurement error of assessing blood pressure, they should be interpreted with caution.

Dietary components most often related to the prevention and treatment of HTN include reduced sodium chloride (salt) and alcohol consumption. Several controlled, dose-response trials, testing at least three sodium levels, have found a statistically significant, direct, and progressive dose-response relationship with blood pressure (Johnson et al. 2001; MacGregor et al. 1989; Sacks et al. 2001). The well recognized Dietary Approaches to Stop Hypertension (DASH)-Sodium trial combined a diet high in fruits, vegetables, and low-fat dairy products with three sodium intakes (50, 100, and 150 mmol/2,100 kcal; 1150 mg, 2300 mg, 3450 mg, respectively) and compared this diet to that of a typical American diet. Blood pressure reduction was direct and progressive in all subgroup categories (i.e., men, women, blacks, nonblacks), at all three sodium levels, and greatest when sodium intake was ≤ 100 mmol/2100 kcal (2300 mg) (Bray et al. 2004). He and Mac Gregor (2002) compiled a meta-analysis documenting that a median reduction in urinary sodium ≈ 1800 mg/d (78 mmol/d) resulted in reduced SBP and DBP, respectively, by 5.0 and 2.7 mmHg in hypertensive individuals and by 2.0 and 1.0 mm Hg in non-hypertensive individuals. The values reported in the non-hypertensive individuals likely lack biological relevance as they fall within the measurement error of blood pressure. Recommendations from the American Heart Association (AHA) suggest reducing salt intake as much as possible, ideally to less than 3.8 g/d sodium (≈ 1500 mg/d) chloride (Appel et al. 2008). Alcohol consumption, independent of age, obesity, and salt intake (Okubu et al. 2001), has been indicated to have a direct, dose-response relationship with blood pressure, especially when consumption exceeds two drinks per day (Klastky et al. 1977; Xin et al. 2001). Recommendations for individuals who drink indicate moderate consumption, limiting men to ≤ 2 drinks per day and women to ≤ 1 drink per day (Appel et al. 2008).

Clinical research trials have indicated that a diet rich in fruits and vegetables, in combination with low-fat dairy products and reduced saturated and total fat, can substantially lower blood pressure (Lawrence et al. 1997). Reduced meat intake has been shown to reduce blood pressure in normotensive and hypertensive individuals, independent of changes in sodium and potassium intake (Rouse et al. 1983). In agreement with clinical trials, epidemiologic studies have shown that individuals who consume a vegetarian diet tend to have lower blood pressures compared to those consuming a nonvegetarian diet (Sacks et al. 1974; Rouse et al. 1983; Appleby et al. 2002). Observational studies have indicated significant inverse relationships between blood pressure and the intake of magnesium, potassium, calcium, and fiber (Ascherio et al. 1992; Harlan et al. 1984). However, clinical trials examining calcium

(Allender et al. 1996), fiber (Eliasson et al. 1992), and other nutrients, most often given as supplements, have found changes in blood pressure to be minimal and inconsistent (Brussaard et al. 1981; Whelton and Klag 1989; Whelton et al. 1992). A meta-analysis that included 16 randomized, controlled trials found a significant rise of 2.0 mmHg in SBP in individuals who consumed caffeine regularly (Noordzij et al. 2005). However, this rise in blood pressure would not be considered biologically important as it falls within the measurement error. This increase in SBP was much smaller in coffee drinkers compared to regular caffeine consumers (caffeinated beverages other than coffee) (Noordzij et al. 2005), suggesting that components in coffee other than caffeine may be involved. A study conducted by Jee et al. (1998) indicated a stronger relationship between coffee and increased blood pressure, whereas Bertrand et al. (1978) concluded that there was no relationship. Finally, cigarette smoking is known to acutely raise blood pressure, whereas epidemiological data have suggested that smokers have lower mean blood pressure compared to nonsmokers (Green et al. 1986), although these findings are inconsistent (Primatesta et al. 2001) and may be confounded by body weight.

Inflammation

Inflammation is an innate and adaptive response triggered by infection and tissue injury. The primary objective of inflammation is to localize, remove, and repair infected or injured tissue. An acute inflammatory response is the initial phase of the inflammatory process, characterized by vasodilation and increased movement of plasma and leukocytes from blood into injured tissue. Tissue-resident macrophages and mast cells recognize the infection and stimulate the production of inflammatory mediators, such as chemokines and cytokines (Medzhitov 2008). These mediators elicit an influx of plasma proteins and leukocytes, primarily neutrophils, to aid in the removal of toxic agents and repair tissue. Neutrophils adhere to the blood vessel wall and extravasate tissue at the site of injury. Once in contact with the afflicted tissue, neutrophils release ROS and hydrolytic enzymes from cytoplasmic granules in an attempt to kill the invader (Nathan 2006). The host tissue is not protected from these potent effectors; host tissue damage occurs simultaneous with repair (Nathan 2002).

If the acute response is unsuccessful in eliminating the toxic agent, chronic inflammation ensues. Leukocytes, lymphocytes, and macrophages replace neutrophils in infiltrating tissue (Medzhitov 2008). In addition to their phagocytic function, macrophages also produce cytokines, prostaglandins, and connective tissue elements. Both macrophages and lymphocytes increase expression of cytokines, which enhance the replication of epithelial cells, fibroblasts, and synthesis of extracellular matrix proteins (Clancy 1998). If continued attempts to engulf and destroy pathogens fail, granulomas form to

wall off foreign bodies (Majno 2004). Granulomas are spherical nodules comprised of organized macrophages that may contain additional immune and matrix cells (Adams 1976). Without regulation or inhibition, these processes can be detrimental to local tissue; connective tissue may accumulate, permanently altering tissue architecture.

Cytokines are immunomodulatory agents vital for signaling and cell communication. By binding to their specific surface receptors, cytokines mediate and alter functional states of cells and tissues (Medzhitov 2008). This change allows cells and tissues to adapt to the inflammatory condition. Cytokines mediate several responses during inflammation, including activation of the endothelium and leukocytes, and prompt the acute-phase response (Medzhitov 2008). Proinflammatory cytokines, specifically tumor necrosis factor- α (TNF- α) and interleukin-1 (IL-1), activate endothelial cells, leading to the production of cell adhesion molecules (CAM) (Schleimer and Rutledge 1986). CAM carry out the site-specific inflammatory response, known as the leukocyte adhesion cascade (**Figure 2**).

Leukocyte Adhesion Cascade

The leukocyte adhesion cascade is a crucial component of the inflammatory response that takes place at the site of injury or inflammation. This complex process secures leukocytes on the endothelium leading to their transendothelial migration. The leukocyte adhesion cascade includes capture, rolling, slow rolling, activation, arrest, adhesion strengthening and spreading, intravascular crawling, and paracellular and transcellular transmigration (Figure 2) (Ley et al. 2007). During inflammation, proinflammatory cytokines IL-1 and TNF- α activate endothelial cells that increase expression of several CAM (Schleimer and Rutledge 1986). CAM are glycoproteins expressed on the surface of leukocytes and various tissue cell types; by binding to their respective ligands, or integrins, on the surface of target cells, they aid in cell-to-cell communication and cell-matrix adhesion (Elangbam et al. 1997). The four primary groups of CAM include the immunoglobulin superfamily, integrin receptor family, selectins, and cadherins (Elangbam et al. 1997).

The selectins family and integrins, very late antigen-4 and P-selectin glycoprotein ligand-1, are involved in the capture and rolling of leukocytes on endothelial cells (Ley et al. 2007). Capture, or tethering, of leukocytes to the endothelium is mediated by L-selectin. The selectin-to-ligand bond allows adhesion of leukocytes to the endothelium despite blood flow (Berlin et al. 1995). In fact, L-selectin and P-selectin require shear stress to bond with their ligands, and bond strength increases as this force is applied (Yago et al. 2007). Rolling is mediated by L-selectin, E-selectin, and P-selectin (Ley et al. 2007). Rolling allows leukocytes to move along the inflamed endothelium at a velocity specific to the

selectin/ligand interaction. L-selectin mediates the fastest rolling velocity, P-selectin a slower velocity, and E-selectin the slowest rolling velocity (Kunkel and Ley 1996). Slow rolling velocities increase transit time of leukocytes on the endothelium, promoting leukocyte activation and arrest (Steeber et al. 2005).

Chemokines or other chemoattractants initiate the arrest of leukocytes on the endothelium (Campbell et al. 1996). Members of the immunoglobulin superfamily, including ICAM-1 and VCAM-1, mediate the arrest and firm adhesion of leukocytes via binding their leukocyte integrins, specifically leukocyte function antigen-1 and very late antigen-4 (Ley et al. 2007). Following arrest, adhesion strength increases and activated leukocytes flatten and spread on the endothelium. As leukocytes spread, extensions of the cell membrane protrude into the endothelial cell-body or junctions. The leukocyte membrane protrusions express high levels of ICAM-1 (Ley et al. 2007). Ligation of ICAM-1 by macrophage antigen-1 triggers the process known as crawling (Schenkel et al. 2004; Phillipson et al. 2006). Crawling allows leukocytes to locate a preferred site for transmigration into the inflamed tissue.

In order to emigrate into the inflamed tissue, leukocytes must pass through three barriers: endothelial cells, endothelial basement membrane, and pericytes. Leukocyte transmigration, also known as diapedesis, can take place via paracellular or transcellular routes. Paracellular migration takes place through endothelial-cell junctions. Transmigration requires an increase of intracellular free calcium concentration in endothelial cells. An intracellular calcium increase activates myosin light chain kinase, leading to endothelial-cell contraction and an opening of interendothelial contacts, aiding in leukocyte passage (Huang et al. 1993). Leukocytes are guided through endothelial junctions via a sequence of homophilic interactions between molecules on the leukocyte and molecules on the endothelium. Several molecules on the endothelium function sequentially to guide the leukocyte through the endothelial junctions. Members of the immunoglobulin superfamily, platelet/endothelial cell adhesion molecule 1 and junctional adhesion molecule A, located near the luminal surface direct leukocytes to endothelial cell junctions. The non-immunoglobulin molecule, CD99, follows the action of platelet/endothelial cell adhesion molecule 1, guiding the leukocyte through the endothelial junctions to the basement membrane.

Transcellular migration involves leukocyte engulfment and transport through thin areas of the endothelium. Ligation of ICAM-1 triggers ICAM-1 translocation and the formation of vesiculo-vacuolar organelles, which create an intracellular channel in the endothelium allowing leukocytes to migrate (Cinamon et al. 2004; Millan et al. 2006). Ezrin, radixin, and moesin proteins may act as a link between cytoskeletal protein and ICAM-1 (Barreiro et al. 2002), together forming 'docking structures' or

'transmigratory cups' to structurally support the cell during transcellular migration (Carman and Springer 2004). It is possible that many of the molecules associated with paracellular transmigration are involved in the engulfment and transport of leukocytes through the endothelium. Following migration through the endothelial barrier, leukocytes penetrate the endothelial basement and the pericyte sheath. During transendothelial migration, leukocytes are often physically altered to aid in further migration (Nourshargh and Marelli-Berg 2005). This migration commonly occurs at regions of low protein density in the matrix and gaps between pericytes (Wang et al. 2006).

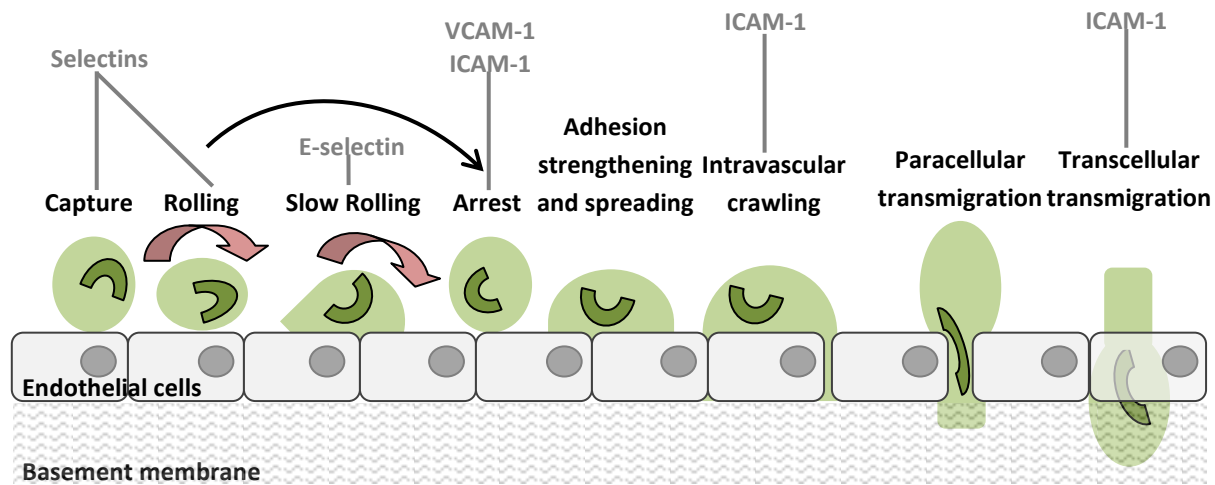


Figure 2. Leukocyte adhesion cascade. E-selectin, Leukocyte-endothelial cell adhesion molecule; VCAM-1, Vascular cell adhesion molecule 1; ICAM-1, Intercellular cell adhesion molecule 1. (Ley et al. 2007)

Cell Adhesion Molecules

Endothelial-leukocyte adhesion molecule-1

E-selectin-1 is a member of the selectins family and is expressed by cytokine-activated endothelial cells. E-selectin binds to the integrin platelet-leukocyte adhesion molecule (P-selectin) glycoprotein ligand-1, which is expressed by neutrophils, monocytes, and lymphocytes (Moore et al. 1995). The primary function of E-selectin is to mediate the capture and slow rolling of leukocytes along the endothelium. In addition to leukocyte capture, E-selectin may also recruit memory T cells to sites of inflammation, primarily inflammation involving the skin (Kansas 1996; Picker et al. 1991). E-selectin expression increases within the first few hours of inflammatory reactions and peaks within 24 hours. However, expression may remain high during chronic inflammatory conditions, such as psoriasis (Kansas 1996).

Vascular cell adhesion molecule-1

VCAM-1 is a member of the immunoglobulin superfamily and is expressed by cytokine-activated endothelial cells (Sano et al. 1995), smooth muscle cells (Ardehali et al. 1995), macrophages (Van Oosten et al. 1995), fibroblasts (Meng et al. 1995), bone marrow stromal cells (Bevliacqua 1993), and neurons (Birdsall et al. 1992). VCAM-1 binds to the integrin very late antigen-4, found on all leukocytes except neutrophils (Elangbam et al. 1997; Elices et al. 1990). In addition to recruiting leukocytes, VCAM-1 mediates the firm adhesion of monocytes, lymphocytes (Carlos et al. 1990), basophils, and eosinophils to endothelial cells (Bochner et al. 1991).

Intercellular adhesion molecule-1

ICAM-1 is a member of the immunoglobulin superfamily and is expressed by cytokine-activated endothelial cells, leukocytes, dermal fibroblasts, and melanocytes (Elangbam et al. 1997; Johnson et al. 1989). ICAM-1 binds to the ligands leukocyte function antigen-1 (CD11a/CD18) and macrophage antigen-1 (CD11b/CD18), found on neutrophils, T cells, and macrophages. Similar to VCAM-1, ICAM-1 aids in the localization and adhesion of leukocytes to areas of inflammation. However, unlike VCAM-1, ICAM-1 participates in neutrophil adhesion to activated endothelium. Beyond its function as an adhesion molecule, ICAM-1 has been characterized recently in playing a role in signal transduction.

Cell Adhesion Molecules, Inflammation, and Atherosclerosis

Inflammation and Atherosclerosis

The array of risk factors contributing to the development of CVD have been described as inter-related processes, characterized by an underlying inflammatory condition leading to atherosclerosis (Ross 1993). These risk factors, including obesity, diabetes, hyperlipidemia, and HTN have been recognized for the chronic inflammatory state they induce (Ross 1993). This inflammatory state persistently activates the endothelium, upregulating the expression of CAM (Schleimer and Rutledge 1986). Recruited leukocytes and monocytes extravasate the endothelium and begin to accumulate lipids, initiating atheroma formation (Zimmerman et al. 1992). Lipid-laden macrophages and smooth muscle cells accumulate within the arterial wall, creating a raised atherosclerotic lesion, thus narrowing blood vessels. With continued narrowing of the blood vessels, myocardial ischemia may occur, leading to angina and myocardial infarction. Calcium eventually collects in atherosclerotic plaques, making them brittle and vulnerable to rupture. The calcified plaque can cause bleeding and thrombi formation, potentially occluding local arteries or detaching to cause an embolism (Demerath et al. 2001).

Factors Related to Cell Adhesion Molecule Expression

Adhesion molecules are shed from the surface of cells and exist in soluble form in circulation, allowing non-invasive quantification of their expression (Rothelin et al. 1991; Gearing et al. 1992). Measuring serum concentrations of soluble adhesion molecules may have a prognostic role in identifying inflammatory conditions that increase the risk of atherosclerotic CVD. The expression of adhesion molecules has been related to age, gender, smoking, cancer, and disease. Increased expression of CAM has been identified in overt CVD and inflammatory (but not preclinical) conditions, such as cancer, obesity, diabetes, hyperlipidemia, HTN, and endothelial dysfunction.

Age

Studies examining rodents have consistently found an increase in the expression of CAM as animals age (Lakatta 2003). However, human studies have found no relationship between soluble adhesion molecules and age, with the exception of a positive association between age and sVCAM-1 (Blann et al. 1996; Demerath et al. 2001; DeSouza et al. 1997; Morisaki et al. 1997; Rohde et al. 1998). The increase in sVCAM-1 may be due to the natural increase in blood pressure that is associated with age, which in turn activates endothelial cells and increases sVCAM-1 expression. The question remains as to why only sVCAM-1 has been noted to increase with age.

Gender

Gender differences in CAM expression are attributed to endogenous production of estrogen and androgen. Estradiol, the most biologically active female hormone, is known to have powerful cardioprotective effects, including promotion of vasodilation, reduction in platelet aggregability, and maintenance of endothelial cell integrity (Nasr and Breckwoldt 1998). In addition, estrogen has been reported to reduce the expression of adhesion molecules *in vitro* (Nasr and Breckwoldt 1998). Androgens have been reported to increase plaque formation in testosterone treated chicks and monkeys. More recently, human umbilical vein endothelial cells exposed to the androgen dihydrotestosterone exhibited increased monocyte adhesion to the endothelium and increased expression of sVCAM-1 (McCrohon et al. 1999). Although sex differences are expected in CAM concentrations, these distinctions have not been consistently reported. Demerath et al. (2001) and Blann et al. (1996) found significantly higher sE-selectin concentration in healthy men compared to healthy women; however, sICAM-1, sVCAM-1, and sP-selectin were not significantly different. It is unknown why a gender difference has only been noted for sE-selectin.

Smoking

Smoking increases the susceptibility of LDL to oxidation (Steinberg 1997), which in turn stimulates endothelial cells and surface expression of CAM (Frostedgard et al. 1993). Smoking has also been shown in hypercholesterolemic individuals to enhance LDL oxidation synergistically, potentiating endothelial dysfunction (Heitzer et al. 1996). Smoking has also been shown to be positively correlated with sICAM-1 (Koundouros et al 1996; Blann et al 1997), sE-selectin, and sP-selectin (Demerath et al. 2001). However, this association was only present in active smokers, since passive smokers and ex-smokers have been shown to have similar CAM concentrations compared with non-smokers (Demerath et al. 2001).

Cancer and other diseases

Adhesion molecules have been implicated in various stages of tumor progression, metastasis, and disease (McCarthy et al. 1991). Banks et al. (1993) found significantly higher sVCAM-1 and sICAM-1 concentrations in patients with various malignancies, including myeloma, Hodgkin's disease, non-Hodgkin's lymphoma, ovarian, breast, gastrointestinal, renal, and bladder cancers compared to healthy controls. Soluble E-selectin was significantly higher in individuals with ovarian, breast, and gastrointestinal cancers compared to controls (Banks et al. 1993). Increased sVCAM-1 has also been found in individuals with chronic inflammatory conditions, such as rheumatoid arthritis and autoimmune diseases (Sasseville et al. 1992), whereas elevated sICAM-1 concentration has been indicated in liver disease.

Obesity

Regardless of blood pressure, obese individuals had higher circulating sICAM-1, sVCAM-1, and sE-selectin concentrations compared to their non-obese hypertensive and normotensive counterparts (Demerath et al. 2001; Ferri et al. 1999). Ferri et al. (1999) reported significant reductions in circulating sICAM-1, sVCAM-1, and sE-selectin following significant BMI reduction (32.9 ± 1.8 to 31.2 ± 2.1 , $P=0.02$) as a result of a 12 week energy-restricted diet. A reduction in weight and/or BMI results typically in loss of adipose tissue. Adipose tissue expresses a variety of secretory proteins, including TNF- α and adiponectin (Arita et al. 1999; Hotamisligil et al. 1993). As mentioned earlier, TNF- α activates endothelial cells to release CAM. Ouchi et al. (1999) reported that adiponectin acted as a beneficial endogenous modulator of endothelial cell function via its suppression of TNF- α action on endothelial cells. Paradoxically, adiponectin concentrations are significantly lower in obese subjects than non-obese subjects, despite adiponectin secretion occurring exclusively from adipose tissue (Arita et al. 1999).

Thus, the lower concentration of plasma adiponectin in obese individuals allows greater TNF- α action on endothelial cells, promoting CAM expression and the leukocyte adhesion cascade. Adiponectin is also known to improve insulin sensitivity (Hotta et al. 2000). The suppressed expression of adiponectin with obesity increases the risk of obese individuals to become insulin resistant and/or diabetic.

Diabetes

Several investigators have found higher concentrations of VCAM-1, ICAM-1, and E-selectin-1 in insulin-dependent diabetic (Fasching et al. 1996) and non-insulin-dependent diabetic individuals (Blüher et al. 2002; Boulbou et al. 2005; Kado and Nagata 1999) compared to healthy individuals (Cominacini et al 1995; Derosa et al. 2010; Steiner et al. 1994). Blüher et al. (2002) evaluated CAM concentration during various glucose and insulin states and found that sICAM-1, sVCAM-1, and sE-selectin were positively correlated with fasting plasma glucose ($r = 0.59$, $P < 0.001$), 2-hour oral glucose tolerance test ($r = 0.70$, $P < 0.01$), and hemoglobin A_{1c} ($r = 0.61$, $P < 0.05$). Unlike sICAM-1 and sVCAM-1, sE-selectin was also correlated with fasting insulin concentration ($r = 0.62$, $P < 0.05$). Individuals with both chronic hyperglycemia and hyperinsulinemia had higher plasma CAM concentrations compared to those with hyperinsulinemia alone. Thus, Blüher et al. (2002) concluded that increased CAM concentration in diabetic individuals was likely a result of chronic hyperglycemia. Individuals with insulin resistance and hyperglycemia (those with Type II diabetes mellitus) are at serious risk of endothelial dysfunction. The chronic periods of hyperglycemia related to insulin resistance lead to the production of advanced glycation endproducts (AGE). Advanced glycation endproducts are known to induce ROS, and thus promote vascular inflammation. In fact, acute hyperglycemia has been shown in humans *in vivo* to reduce nitric oxide and impair endothelium-dependent vasodilation (Williams et al. 1998).

Hyperlipidemia

Hyperlipidemia causes activation of the endothelium, stimulating the release of CAM and the leukocyte adhesion cascade. Excess LDL in the circulation promotes infiltration of LDL in the intimal layer of the artery, signaling an inflammatory response. In addition, LDL may undergo oxidation or enzymatic damage, further stimulating the production of CAM. Individuals with high triglycerides (911 ± 111 mg/dL) compared to controls (97 ± 7 mg/dL) had significantly higher sVCAM-1 and sICAM-1 concentrations (Hackman et al. 1996). Soluble ICAM-1 concentration was found to be significantly higher in individuals with high total cholesterol (345 ± 16 mg/dL) compared to controls (201 ± 9 mg/dL). Individuals with atherosclerosis in addition to hyperlipidemia had a significantly higher concentration of sVCAM-1 (977 ± 143 vs 566 ± 37 ng/mL, $P \leq 0.0004$) compared to individuals with hyperlipidemia alone

(Hackman et al. 1996). Soluble ICAM-1 and sVCAM-1 concentrations were also significantly higher in individuals who exhibited hyperlipidemia plus any other risk factor (Hackman et al. 1996).

Hypertension

The relationship between HTN and CAM may be a key component to the increased risk of atherosclerotic CVD associated with HTN. Individuals with essential HTN were reported to have higher serum sICAM-1 (Chae et al. 2001; DeSouza et al. 1997), sVCAM-1 (DeSouza et al. 1997), and sE-selectin (Blann et al. 1994) concentrations compared to normotensives (Buemi et al. 1997). Even during acute elevations in blood pressure, serum concentrations of sICAM-1, sVCAM-1, and E-selectin increased in both normotensive and hypertensive individuals (Buemi et al. 1997). A cross-sectional study of 508 healthy men found that increases in SBP were significantly associated with increased sICAM-1 concentration, whereas increases in both SBP and DBP were significantly associated with increased IL-6 concentration, a proinflammatory cytokine (Chae et al. 2001). As described previously, reduced circulating adiponectin has been associated with greater CAM expression. Adamczak et al. (2003) reported significantly lower adiponectin concentration in hypertensive individuals compared to age-, gender-, and BMI-matched normotensive individuals. Adamczak et al. (2003) also demonstrated a significant negative correlation between adiponectin and blood pressure. The increased expression of adhesion molecules associated with HTN may explain the relationship between HTN and endothelial dysfunction.

Endothelial dysfunction

Endothelial dysfunction refers to a condition of reduced vasodilation, which coincides with a proinflammatory and prothrombotic state (Endemann and Schiffrin 2004). The impairment of vasodilation has been indicated in metabolic syndrome and dyslipidemia (Engler et al. 2003), type 1 diabetes (Bechman et al. 2003), type 2 diabetes (Rizzoni et al. 2001; Schofield et al. 2002), coronary artery disease (Monnink et al. 2002), congestive heart failure (Lendmesser et al. 2002), and chronic renal failure (Bolton et al. 2001; Thambryajah et al. 2000). Endothelial dysfunction may not only be related to existing CVD, but also as a component of its development.

Potential risk factors for endothelial dysfunction include obesity (Raitakeri et al. 2004), hyperhomocysteinemia (Virids et al. 2001), physical inactivity (Green et al. 2003), and smoking (Oida et al. 2003). The mechanisms related to the pathophysiology of endothelial dysfunction are complex, and commonly involve nitric oxide, oxidative excess, and inflammation (Endemann and Schiffrin 2004). Hypertensive individuals often exhibit oxidative excess, leading to diminished nitric oxide production

and endothelial dysfunction (Endemann and Schiffrin 2004; Taddei et al. 1998). Oxidative excess limits reductase function, causing the formation of excess ROS. Reactive oxygen species upregulate the expression of adhesion molecules VCAM-1 and ICAM-1, as well as other chemotactic molecules, promoting a proinflammatory state within the endothelium (Endemann and Schiffrin 2004; Griendling and FitzGerald 2003).

Hypertensive individuals have an elevated concentration of Ang II, also implicated in the pathophysiology of endothelial dysfunction. Ang II infusion has been shown to increase ROS and to promote vascular inflammation, leading to endothelial dysfunction (Rajagopalan et al. 1996; Touyz and Schiffrin 1999). Administration of Ang II directly stimulated VCAM-1 (Pueyo et al. 1998) and ICAM-1 (Pastore et al. 1999) expression in endothelial cells, and E-selectin expression in coronary endothelial cells (Grafe et al. 1997). Expression of VCAM-1 may be induced or mediated by the redox-sensitive transcription factor nuclear factor- κ B (NF- κ B) (Collins et al. 1995). Ang II activates NF- κ B in endothelial cells (Pueyo et al. 1998), and NF- κ B in turn regulates an inflammatory and proliferative cell response (Brand et al. 1996), which has been identified in endothelial cells and atherosclerotic tissue (Brand et al. 1996). These mechanisms suggest that increases in adhesion molecule expression are not only a direct, but also indirect, consequence of HTN.

Cardiovascular disease

Adhesion molecules have been implicated in the progression and consequences of atherosclerotic CVD. Soluble ICAM-1 (Rohde et al. 1999) and sVCAM-1 (DeCaterina et al. 1997; Rohde et al. 1998) have been identified as markers of the severity of carotid atherosclerosis, and in addition to sE-selectin, have been detected in human atherosclerotic lesions (Davies et al. 1993). Unlike sVCAM-1, sICAM-1 has been found to be an independent risk factor for myocardial infarction (Hwang et al. 1997; Ridker et al. 1998) and has consistently been associated with established CVD risk factors in males (Demerath et al. 2001; Rohde et al. 1999). Blann and McCollum (1994) also found significantly higher concentration of sICAM-1, but not sVCAM-1, in patients with ischemic heart disease and peripheral vascular disease compared to that of age-matched control subjects. Elevated sE-selectin expression has been reported following ischemic stroke (Frinhs et al. 1997) and disseminated intravascular coagulation. The elevated expression of CAM in chronic inflammatory conditions, as well as overt CVD, suggests that adhesion molecules may serve as markers of atherosclerosis and the consequent development of CVD (Hwang et al. 1997). The reduced expression of CAM may indicate suppressed inflammatory activity, with a subsequent decreased risk of atherosclerotic CVD. Identifying clinical interventions that may

reduce CAM concentrations in high risk individuals, such as hypertensives, may also provide an avenue to secondary prevention of atherosclerosis.

Milk Beverage Intervention

Hypertensive individuals are at an increased risk of inflammation and atherosclerotic CVD. Furthermore, CAM concentrations are typically elevated in persons with HTN, inflammation, and CVD. A reduction in blood pressure or CAM concentrations may reduce the risk of atherosclerotic CVD via modulation of the inflammatory state. Previous investigations have indicated an inverse relationship between milk, either cow or soy, and blood pressure. Identifying a dietary intervention to reduce CAM concentrations in at-risk individuals may provide a secondary preventive measure for atherosclerotic CVD.

Cow's Milk

Blood pressure response

The relationship between blood pressure and dairy intake was reported in the first National Health and Nutrition Examination Survey (NHANES I) analysis, in which a cross-sectional study of greater than 10,000 individuals revealed that low milk consumption was related to increased prevalence of HTN (McCarron et al. 1984). A study of Puerto Rican men reported that those who did not drink milk had a two-fold greater prevalence of HTN compared to men who drank at least 1 L milk/day (Farcia-Palmieri et al. 1984). The relationship between HTN and reduced milk intake has also been reported in American (Ackley et al. 1983) and Italian (Trevisan et al. 1988) population studies. One large observational study of university graduates indicated that low-fat dairy consumption, but not whole-fat dairy consumption, was associated with a lower risk of incident HTN (Alonso et al. 2005).

Following these and other epidemiological reports, intervention studies investigated the impact of dairy consumption on blood pressure. The well recognized DASH trial examined a diet rich in fruits, vegetables, and low-fat dairy products consumed by normotensive and hypertensive subjects. Mean blood pressure was significantly reduced in both normotensive and hypertensive subjects (mean SBP by 5.5 mmHg and DBP by 3.0 mmHg); however, blood pressure reduction (mean SBP by 11.4 mmHg and DBP by 5.5 mmHg) in the hypertensive subjects was greater than in the normotensive subjects (Appel et al. 1997; McCarron and Reusser 1999). Milk supplementation has been shown to reduce blood pressure in several studies. Buonopane et al (1992) reported a significant reduction in SBP and DBP in adults who were supplemented with one quart of skim milk per day for eight weeks. Hilary et al. (2000) examined the effect of a fourweek supplementation of skim, high calcium skim, or potassium-enriched high

calcium skim milk on blood pressure in healthy subjects greater than 40 years of age. Seated SBP was significantly reduced in all treatment groups; however, there was no change in DBP. The greatest reduction in SBP was in the potassium-enriched high calcium skim milk group (Hilary et al. 2000). In another study, a significant reduction in SBP (6.8 mmHg, $P < 0.01$) and DBP (4.3 mmHg, $P < 0.01$) was observed for obese African American individuals supplemented with a high dairy calcium diet (3 servings per day) compared to a low dairy calcium diet (<1 serving per day) (Zemel et al. 2005). Although evidence strongly suggests a relationship between dairy intake and reduced blood pressure, this has not been demonstrated in all studies. Healthy older adults (55-85 years of age) who were advised to increase skim or 1% milk consumption by 3 cups per day did not exhibit a greater reduction in blood pressure compared to matched controls who did not change their diet (Barr et al. 2000). One study found that supplementation with whole-fat milk actually increased significantly SBP, but not DBP, in young normotensive adults (18-24 years of age) (Alonso et al. 2009).

Initially, it was assumed that dairy calcium was responsible for the inverse relationship between dairy consumption and blood pressure. Research has shown that individuals with HTN have reduced serum ionized calcium (McCarron 1982) and non-dairy calcium supplements also have been effective in reducing blood pressure (Belizan et al. 1983; Dwyer et al. 1998). Yet, additional investigations have found that the proteins in milk, casein and whey, either as whole proteins or their degradation products, also contribute to the blood pressure-reducing effect of dairy products. Pal and Ellis (2010) recently investigated changes in blood pressure in overweight or obese subjects supplemented with whey protein isolate, sodium caseinate, or a glucose control, which they consumed with 250 mL of water, two times daily for 12 weeks. In the whey and casein groups, SBP was significantly reduced at 6 and 12 weeks compared to baseline SBP, while DBP was significantly reduced at 12 weeks compared to baseline. Although in the treatment groups blood pressure was significantly reduced compared to baseline values, only the reduction of DBP was significantly different among the treatment and control groups.

Attention has recently turned toward milk-derived bioactive peptides, the degradation products of proteins. These peptides are released during food processing or digestion and have been credited with a number of physiological functions including antihypertensive, immunomodulatory, and antioxidative properties (Jäkälä and Vapaatalo 2010). The greatest interest has focused on particular tripeptides released during fermentation of milk with specific lactobacilli strains (Jäkälä and Vapaatalo 2010). The primary milk-derived bioactive peptides are casein-derived Ile-Pro-Pro and Val-Pro-Pro

(Jäkälä and Vapaatalo 2010). These tripeptides have been shown to inhibit angiotensin-converting enzyme *in vitro* (Nakamura et al. 1995) and have been effective in reducing blood pressure in humans (de Leeuw et al. 2009; Nakamura et al. 2009; Yoshizawa et al. 2009). Two recent meta-analyses examined blood pressure response to peptides and found similar results. Xu et al. (2008) found significant decreases of 4.8 mmHg in SBP and 2.2 mm in DBP; likewise, Pripp (2008) found significant decreases of 4.6 mmHg in SBP and 2.2 mmHg in DBP. Nearly all clinical trials in this area have studied the long-term effects of lactotripeptides on blood pressure. However, van der Zander et al. (2008) observed an acute and significant reduction of 2 mmHg in SBP following ingestion of a lactotriptide-containing milk product. After several long-term studies examining these effects, researchers have found that supplementing pure tripeptides does not produce as strong an antihypertensive effect as the milk products themselves (Jauhianinen et al. 2005; Sipola et al. 2001). It is important to note that although changes in blood pressure have been found to be statistically significant, the actual value is relatively small and cannot be evaluated independent from measurement error, casting particular doubt on the biological relevance for DBP reduction.

Cow's milk may modulate blood pressure through several of its components: the proteins, minerals (high in calcium, phosphorus, potassium, selenium), or the combination of both in a milk product. Cow's milk consumption may be a relatively simple potential clinical intervention to reduce blood pressure, thereby reducing inflammation and the risk of atherosclerotic CVD. Provided cow's milk consumption results in a simultaneous reduction in blood pressure and in CAM concentration, the risk of atherosclerotic CVD might be further reduced with this dietary intervention.

Cell adhesion molecule expression

Little research has been conducted on the effect of dairy consumption on inflammatory markers, such as CAM. In theory, the relationship between high blood pressure and increased CAM expression would suggest that a reduction in blood pressure following dairy consumption would, in turn, reduce CAM. Wennersberg et al. (2009) investigated the effects of an increased intake of dairy products on several factors in overweight individuals who typically consume a low amount of dairy foods. Individuals who consumed 3-5 servings of dairy products daily did not exhibit any significant changes in sVCAM-1 concentration compared to the control group, although when sexes were examined separately, sVCAM-1 was significantly ($P = 0.001$) reduced in women who increased their intake. A crossover study of 486 adult Iranian women (40-60 years of age) collected dairy intake data based on a food frequency questionnaire and examined markers of inflammation, including sVCAM-1

concentration. Low-fat dairy intake was inversely correlated to sVCAM-1; however, high-fat dairy intake was positively correlated to sVCAM-1 (Esmailzadeh and Azadbakht 2010), illustrating that the fat content of milk may be a critically important determinant of the inflammatory response. These controversial results, along with the limited number of studies in this area of research, indicate that further investigation to verify a relationship between cow's milk and CAM concentrations is needed.

Soy Products

Blood pressure response

Soy foods have been suggested to improve CVD-related risk factors, and considerable interest has been given to their potential hypocholesterolemic properties (Sacks et al. 2006; Zhan and Ho 2005). Two characteristic components of soy products are the high-quality soy protein and bioactive molecules associated with the protein, particularly the isoflavones. Isoflavones have been reported to exert several physiological responses in animals, including arterial vasodilation (Anthony et al. 1998), serum cholesterol reduction (Anthony et al. 1998), and atherosclerosis inhibition (Clarkson et al. 2001). Investigators have often used soy protein supplements without considering the isoflavone content or the preparation of the soy protein, which may partially explain the wide variability in results. The isoflavone content of the product depends heavily upon the processing. If the protein is extracted with an alcohol wash, as might be the case with soy protein isolate, a substantial amount of isoflavones will be lost (Sacks et al. 2008). The three primary isoflavones (in aglycone form) found in soybeans are genistein, daidzein, and glycitein; the quantity of each in soy products differs greatly due to the variety of processing techniques (Sacks et al. 2008). Since the late 1990s, most published studies state the isoflavone content (form and dose) provided to participants, since investigators consider these particulars as important variables that can markedly affect the outcome.

A number of studies have been conducted to examine the influence of soy protein consumption on CVD-related risk factors, including blood pressure. However, the subjects are often postmenopausal women, the amount of soy protein and isoflavones vary greatly, and the results are quite inconsistent. A cross-sectional study in Japanese men and peri- or postmenopausal women evaluated the relationship between estimated soy product intake and blood pressure. Soy product intake was negatively correlated in men to DBP ($r = -0.12$, $P = 0.04$) and to SBP ($r = -0.10$, $P = 0.09$), whereas no significant correlations were found in women (Nagata et al. 2003). He et al. (2005) supplemented normotensive and hypertensive men and women in the People's Republic of China with isolated soybean protein (40 g/day) incorporated into cookies for 12 weeks. The soy protein contained 76.4 mg of total isoflavone

(44.9 mg genistein, 26.5 mg daidzein, 4.9 mg glycitein [aglycone units]). Among the supplemented group, SBP and DBP were reduced by 7.9 mmHg and 5.3 mmHg, respectively, for the hypertensive individuals, whereas were reduced by 2.3 mmHg and 1.3 mmHg, respectively, for the nonhypertensive individuals (He et al. 2005). Using a crossover design, Jenkins et al. (2002) provided hyperlipidemic men and postmenopausal women with a low fat control, a high isoflavone (50 g soy protein and 73 mg isoflavones), and a low isoflavone (52 g soy protein and 10 mg isoflavones) diet, each daily for one month period. Blood pressure reduction only occurred in men on the high isoflavone diet compared to control, whereas no significant difference was noted between the high- and low-isoflavone diets. Men and postmenopausal women supplemented with 28 g soy protein isolate powder (19.9 g protein, 42.0 mg isoflavone [26.8 mg genistein, 13.1 mg daidzein, 1.8 mg glycitein]) twice daily for 3 months experienced a significant decrease in mean blood pressure, SBP, and DBP compared to the control group (Teede et al. 2001). In the supplemented group, mean reductions in SBP and DBP were 7.5 and 4.3 mmHg, respectively, compared to the 3.6 and 1.9 mmHg, respectively, in the casein control group. The most dramatic results were reported by Rivas et al. (2002), in which men and women with mild to moderate HTN were supplemented with 500 mL of a high isoflavone soy milk (80 mg genistein and 63 mg daidzein per 500 mL) twice per day for three months. After supplementation, SBP and DBP, respectively, were significantly reduced in the soy milk group (18.4 ± 10.7 mmHg, $P < 0.0001$ and 15.9 ± 9.8 mmHg, $P < 0.0001$) compared to the cow's skim milk group (1.4 ± 7.2 mmHg and 3.7 ± 5.0 mmHg).

Although the evidence presented herein suggests a relationship between soy product intake and reduced blood pressure, several studies investigating soy protein or soy isoflavones and blood pressure have reported no effect (Hermansen et al. 2001; Hodgson et al. 1999; Nestel et al. 1997; Simons et al. 2000). Teede et al. (2006) supplemented hypertensive men and postmenopausal women with soy cereal (40 g protein, 118 mg total isoflavones) for 6 months and examined the 24 hour blood pressure response. Mean blood pressure during 24 hours did not change within or between soy and placebo groups. However, five years prior, Teede et al. (2001) supplemented healthy men and postmenopausal women for 3 months with the same soy cereal and examined single blood pressure measurements. Reductions in mean SBP and DBP, respectively, were significantly greater in the soy milk (7.5 mmHg and 4.3 mmHg) compared to the placebo (3.5 mmHg and 1.9 mmHg) group.

Despite apparently conflicting results, after compiling 14 randomized controlled studies, the most recent meta-analysis examining soy isoflavone extract supplements and blood pressure in normotensive or prehypertensive adults found a significant overall reduction in SBP of 1.9 mmHg, but

not DBP, in the soy isoflavone compared to placebo controlled treatment. Daily isoflavone supplements varied between 25 and 375 mg total isoflavones and duration ranged from 2 to 24 weeks. Treatment had the greatest effect in studies lasting longer than 3 months (Taku et al. 2010). However, this small change in SBP calculated from previously published studies would be near impossible to detect given measurement error, thus casting doubt on its biological relevance. Soy protein may reduce blood pressure through a variety of its components: the proteins, its isoflavones, other bioactive components, or the combination of bioactive components and protein in soy products. The question becomes whether or not the soy protein effect is sufficiently robust to produce a meaningful reduction in blood pressure in most individuals. If it is, soy product intake may be a relatively simple dietary intervention to reduce blood pressure, thereby reducing inflammation, CAM concentration, and the risk of atherosclerotic CVD.

Cell adhesion molecule expression

A decrease in blood pressure and/or serum cholesterol reduces overall CVD risk. A direct measure of inflammatory status at the molecular level may be CAM expression. Research has shown that soy product consumption may be associated with a reduction in blood pressure and serum cholesterol. A concurrent or independent reduction in adhesion molecule expression following soy intake would further support the relationship between soy consumption and decreased CVD risk.

The isoflavones found in soy foods, genistein, daidzein, and glycitein (and their respective glycosides), have been reported to have anti-inflammatory properties (Chacko et al. 2005; Wei et al. 1995). Atteritano et al. (2007) supplemented postmenopausal women with 54 mg/day of genistein for 2 years and found sVCAM-1 and sICAM-1 to be significantly ($P < 0.05$) reduced compared to placebo. Colacurci et al. (2005) supplemented postmenopausal women with an isoflavone tablet and also reported a significant reduction in CAM, including sVCAM-1, sICAM-1, and E-selectin. Postmenopausal women with metabolic syndrome consumed a DASH diet in addition to replacing one serving of red meat with 30 g of soy protein (281 mg total isoflavones) or 30 g soy nuts (340 mg total isoflavones). The female subjects exhibited a significant reduction in sE-selectin concentration in response to both diets compared to the DASH diet alone (Azadbakht et al. 2007).

Although some studies agree there is a relationship between soy product intake and reduced CAM concentrations, others have not reported these same results. Steinberg et al. (2003) investigated the effects of 25 g soy protein isolate 3 times per day with high isoflavones (107 mg total isoflavones), 25 g ethanol-washed soy protein isolate with low isoflavones (2 mg total isoflavones), and 25 g milk

protein (no isoflavones) for 6 weeks on endothelial function in healthy postmenopausal women. No change was observed among the three treatment groups. Nikander et al. (2003) also supplemented postmenopausal women with soy isoflavones (114 mg/day), reporting no significant difference in CAM between treatment and placebo groups. However, serum daidzein ($r = 0.39$; $P < 0.003$) and genistein ($r = 0.38$; $P < 0.004$) concentrations were inversely associated with E-selectin concentration (Nikander et al. 2003).

A potential explanation for the cardioprotective effect of soy isoflavones is that they exert activity similar to that of estrogen and have particular affinity for estrogen receptor β . Estrogen enhances nitric oxide production, modulating vasodilatory responses and improving endothelial function (Hall et al. 2005). Although isoflavones do not exert the same potency as estrogen, isoflavone intake can elevate circulating isoflavone concentrations up to 10,000 fold greater than that of endogenous estrogen (Aldercreutz et al. 1993). In addition, genistein has been shown to have a potent anti-inflammatory action by inhibiting monocyte adhesion to cytokine-activated endothelial cells (Chako et al. 2005).

In addition, the variable results in response to soy products may be due to the ability of individuals to produce equol, an endogenous degradation product of daidzein, which has been implicated as a highly bioactive metabolite. However, intestinal bacteria that degrade daidzein to equol are evidently not universally present in all individuals (Bingham et al. 2003); most studies have indicated that ~30% of individuals produce equol. Studies also vary greatly in the amount of isoflavones and soy protein provided to subjects, in addition to different lengths of supplementation time. Perhaps the greatest difference among studies is with respect to subject characteristics. For example, a majority of published research using soy protein and isoflavones has been almost exclusively conducted in postmenopausal women. These results also have been inconsistent, perhaps because of differences in ethnicity, age, time since menopause, baseline blood pressure, or unknown host factors. Nevertheless, the effect of soy protein and isoflavones on CAM in men and premenopausal women at-risk for atherosclerotic CVD remains relatively unexplored.

Summary

Inflammation is related to both HTN and CAM, suggesting that the inflammatory process is central to an increased risk of atherosclerotic CVD. Cow's milk and soy product consumption perhaps have the potential to decrease inflammation and hence attenuate the risk of atherosclerotic CVD, as evidenced by a reduction in blood pressure and circulating CAM concentrations in selected subjects

from some but not all studies. Further research is warranted to determine the extent to which cow's milk or two preparations of soy milk (with different levels of isoflavones) may favorably affect blood pressure and/or adhesion molecules in men and premenopausal women at risk of atherosclerotic CVD because of their prehypertensive/Stage 1 hypertensive state.

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The Effect of Soy Protein Beverages on Serum Cell Adhesion Molecule Concentrations in Prehypertensive/Stage 1 Hypertensive Individuals

A paper to be submitted to Nutrition, Metabolism and Cardiovascular Diseases

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ABSTRACT

Background and Aims: Prehypertensive and hypertensive individuals are at an increased risk of atherosclerotic cardiovascular disease. The role of hypertension in endothelial dysfunction and increased cell adhesion molecule (CAM) expression may lead to atherosclerotic progression. Soy protein and isoflavones have been shown to favorably alter cardiovascular disease risk factors. The aim of this study was to determine the effect of daily cow's milk compared with soy beverage prepared from whole soy bean (WSB) or soy protein isolate (SPI) on soluble cell adhesion molecules.

Methods: We enrolled healthy prehypertensive/Stage 1 hypertensive men (n=60, aged 18–63 yr) and premenopausal women (n=8, aged 20–48 yr) and randomized them to one of three beverage groups for 8 weeks of treatment: cow's milk (600 mL/d), soy beverage (840 mL/d) prepared from SPI (30.1 mg total isoflavones/d [aglycone form]) or prepared from WSB (91.4 mg total isoflavones/d [aglycone form]). We measured soluble vascular cell adhesion molecule-1 (VCAM-1), intercellular cell adhesion molecule-1 (ICAM-1), and endothelial-leukocyte adhesion molecule-1 (E-selectin) concentrations at baseline and week 8.

Results: Treatment did not alter soluble CAM concentrations. Time had an effect on VCAM-1 (-9%, $P = 0.01$) and E-selectin (+4%, $P = 0.01$) but not ICAM-1 (+5%, $P=0.86$). When included as a covariate, gender had a significant effect on ICAM-1 ($P=0.0037$), whereas gender did not reach significance for E-selectin ($P=0.067$) or VCAM-1 ($P=0.16$). However, men had higher circulating concentrations of ICAM-1 and E-selectin, respectively, at both baseline ($P = 0.0071$, $P = 0.049$) and week 8 ($P = 0.0054$, $P = 0.038$) than women. ICAM concentrations were not significantly different between prehypertensive and hypertensive participants.

Conclusion: Prehypertensive/Stage I hypertensive individuals who consumed either cow's milk or soy beverages (prepared from WSB or SPI) for 8 weeks daily did not show any change in soluble CAM concentrations. Consequently, we cannot suggest that daily intake of either cow's milk or soy protein beverages improves circulating CAM concentrations and hence risk of atherosclerotic CVD in these individuals.

KEY WORDS: Cardiovascular disease risk, Cell adhesion molecules, VCAM-1, ICAM-1, E-selectin, Hypertension, Isoflavones, Soy protein

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INTRODUCTION

Atherosclerotic cardiovascular disease (CVD) is the number one cause of death in the United States, accounting for more than 50 percent of all mortality (Lloyd-Jones et al. 2010). Ross (1999) described CVD as a condition of underlying inflammation, leading to the progression of atherosclerosis. Conditions implicated in perpetuating chronic inflammation include diabetes, obesity, hyperlipidemia, and hypertension (HTN) (Ross 1999). HTN is a primary, yet modifiable, risk factor for atherosclerotic CVD. In the US, 29 percent of the adult population has HTN, in addition to 37 percent who have prehypertension (Ostchega et al. 2008). An increase in angiotensin II concentration (Ross 1999), as well as a reduction in both nitric oxide (Endemann and Schiffrin 2004; Taddei et al. 1998) and adiponectin production (Adamczak et al. 2003), are related to HTN. HTN also increases shear stress on the vessel wall; endothelial cells in turn modify their morphology, gene expression, and function in response to shear stress (Ando & Yamamoto 2009). The chemical alterations and mechanical damage associated with HTN persistently activate endothelial cells, contributing to endothelial dysfunction. An excess of prothrombotic and proinflammatory signals coincide with endothelial dysfunction, promoting the expression of chemotactic molecules and cytokines, particularly tumor necrosis factor- α (TNF- α) and interleukin-1 (IL-1) (Endemann and Schiffrin 2004; Griendling and FitzGerald 2003). In response to these cytokines, activated endothelial cells express cell adhesion molecules (CAM), specifically vascular cell

adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and endothelial-leukocyte adhesion molecule-1 (E-selectin) (Schleimer and Rutledge 1986).

These CAM are glycoproteins located on the surface of a variety of cells, mainly endothelial cells, and aid in cell-cell communication and cell-matrix adhesion (Elangbam et al. 1997). By binding to their respective integrins, or ligands, CAM perform the site-specific inflammatory response known as the leukocyte adhesion cascade (Ley et al. 2007). This process controls the locomotion and migration of leukocytes through the endothelium where they repair injured tissue or remove toxic agents. During chronic inflammatory states, such as HTN, this process may continue unregulated. Leukocytes, foam cells, and smooth muscle cells accumulate and proliferate in the subendothelial space forming a lesion (Ross 1999). The enlarging lesion may completely occlude the blood vessel, or collect calcium forming a plaque. An atherosclerotic plaque weakens the vessel wall, making it vulnerable to rupture. Ultimately this process may lead to myocardial infarction or stroke (Demerath et al. 2001; Ross 1999).

Due to several mechanisms by which HTN contributes to endothelial dysfunction, hypertensive individuals likely express higher CAM concentrations, thus placing them at greater risk of atherosclerotic CVD. Soy isoflavones, bioactive molecules structurally similar to 17 β -estradiol, have been hypothesized to protect against atherosclerotic CVD (Sacks et al. 2006; Zhan and Ho 2005). Moreover, clinical studies have reported that soy isoflavones increase nitric oxide production, thereby increasing arterial vasodilation (Anthony et al. 1998) and independently reducing CAM concentrations (Endemann and Schiffrin 2004; Griendling and FitzGerald 2003). A reduction in CAM expression may suppress the leukocyte adhesion cascade, thereby reducing accumulating leukocytes and potentially atherosclerotic progression. Prior investigations have indicated that hypertensive individuals have significantly greater concentrations of CAM compared to normotensive individuals (Blann et al. 1994; Buemi et al. 1997; Chae et al. 2001; DeSouza et al. 1997). However, much of the research examining the effects of soy food intake on these markers investigated hypercholesterolemic postmenopausal women. Therefore, the aim of this study was to determine the effect of soy beverage consumption versus cow's milk on CAM concentrations in otherwise apparently healthy prehypertensive/Stage 1 hypertensive men and women. We hypothesized that soy beverages (either prepared from WSB or SPI) would reduce CAM concentrations in prehypertensive/Stage 1 hypertensive individuals to a greater extent than cow's milk, regardless of whether or not blood pressure was impacted. Further, we anticipated that the soy beverage prepared from WSB, due to its higher isoflavone content, would reduce CAM concentrations to a greater extent than the soy beverage prepared from SPI.

METHODS

Research Design

This study was designed to determine whether drinking soy beverage versus cow's milk, daily for eight weeks would reduce circulating concentrations of sICAM-1, sVCAM-1, and sE-selectin. Blood and 24-hour urine samples were collected at baseline and at week 8. Resting blood pressure was measured at baseline, 2, 4, 6, and 8 weeks. Ambulatory blood pressure was measured at baseline, 4, and 8 weeks. The study protocol, consent form, and subject-related materials were approved by the Institutional Review Boards (IRB) at Iowa State University (ID 02-199). We obtained informed consent from all individuals at the initial screening and after screening criteria were met.

Subject Screening, Selection, and Characteristics

We recruited subjects throughout the Ames, IA region primarily through mass email listings, local newspaper advertisements, and the distribution of informative flyers. Those who responded were screened using a questionnaire to identify healthy individuals who were premenopausal women 18-50 years of age or men 18-65 years of age, not on any medication for HTN, and had not taken antibiotics within the last month. Participants who gained or lost more than 10 lbs in the past six months or had a body mass index (BMI, kg/m²) above 35 were excluded. Vegans, vegetarians, and those on special diets to lose weight or control sodium intake were excluded. We also excluded individuals with allergies or intolerances to milk or soy protein and those who were habitual soy product consumers. Additionally, we excluded pregnant or nursing mothers, peri- and postmenopausal women, or women who had a complete hysterectomy. A pregnancy test was completed during the screening of each female participant.

During the initial screening visit we measured 10-minute resting blood pressure (IntelliSense Professional HEM-907XL Digital Blood Pressure Monitor; Omron Healthcare Inc., Bannockburn, IL), height, weight, and waist circumference. Individuals with a SBP greater than 120 and/or DBP greater than 80 were included in the study. If SBP was greater than 160 and/or DBP was greater than 100, we required written permission from the participant's physician. A randomized, single-blind taste test of the three milk beverages was conducted at the end of the initial screening appointment. To ensure minimal acceptance of treatment beverage and an ability to randomize to at least two treatments, individuals were excluded if he or she rated two or more of the three treatment beverages below 4 on a 1-7 scale (1: terrible, 4: acceptable, 7: delicious), or ≥ 2 of the 3 beverages were marked that the individual would not agree to consume 3.5 cups every day for 2 months.

Participants who met the initial screening criteria were invited to the second screening during which a licensed phlebotomist collected fasted blood samples for a metabolic profile. Blood glucose, total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, blood urea nitrogen (BUN), creatinine, and liver enzymes were required to fall within the reference ranges provided by the certified clinical laboratory (LabCorp; Kansas City, MO). If this was not the case, we required written permission from the participant's physician.

Subject Randomization and Treatment

Qualified participants were randomly assigned to one of three groups: cow's milk (n=21); soy beverage prepared from WSB (n=23); soy beverage prepared from SPI (n=24). The composition of the three treatment beverages is presented in **Table 1**. The beverages were matched closely for energy, carbohydrate, protein, fat, and sodium content. Soy beverage isoflavones were extracted and analyzed by HPLC (Beckman Coulter; Fullerton, CA) at Iowa State University (Murphy et al. 1981). The WSB beverage contained a greater amount of all isoflavones compared to the SPI beverage (Table 1). Subjects in the cow's milk group consumed 2.5 cups/day while limiting other dairy product intake to ≤ 1 serving/day to ensure comparability with the soy beverage groups. Subjects in either of the soy beverage groups consumed 3.5 cups/day while asked to consume no other soy products and limit dairy product intake to ≤ 1 serving/day. Participants were asked to otherwise maintain their normal diet (with the exception of dairy product and soy product intake) and physical activity for the duration of the study. As one check on compliance, they were also asked to return each week all unused treatment beverages in their original containers.

Data Collection

Urinary Measurements

At baseline and week 8, each subject was provided explicit directions for collecting 24-hour urine samples, which were measured and recorded for total volume. Aliquots were stored at -20°C . Concentrations of urinary sodium and potassium were analyzed by a certified clinical laboratory (LabCorp; Kansas City, Kansas). Extracted urinary isoflavones were analyzed by HPLC (Beckman Coulter; Fullerton, CA) at Iowa State University (Lundh et al. 1988; Murphy et al. 1997; Zhang et al. 1999). In addition to returning unused beverage containers each week, urinary isoflavone excretion was used to corroborate treatment adherence (**Table 2**).

Body Size and Composition Assessment

A trained research assistant assessed anthropometric measures for each subject. During the initial screening visit, height, weight, BMI, and waist circumference measurements were taken. Height was measured (to the nearest 0.1 cm) without shoes with a wall-mounted stadiometer (Ayrton stadiometer, Model S100; Ayrton Corp., Prior Lake, MN). Body weight was measured (to the nearest 0.1 kg), with each subject wearing light clothing without shoes, using an electronic scale (Detecto Digital Weight Indicator, Model 768C; Cardinal Scale Manufacturing Co., Webb City, MO). Weight and height measures were used to calculate BMI (**Table 3**). Waist circumference at the natural waist line for each woman and one inch above the naval for men was measured with a tape measure (non-stretchable) while subjects stood relaxed with hands at their sides.

Questionnaires and Dietary Intake Assessment

At the initial screening visit each participant completed a screening questionnaire and a soy food questionnaire (Kirk et al. 1999). We excluded potential participants who indicated that they consumed whole soybean foods or foods containing soy protein more than once per month. Following the screening visits, potential participants also completed a medical history questionnaire.

Dietary intake was assessed at baseline with the use of a three-day food diary. Participants were asked to record everything consumed during two consecutive weekdays followed by a weekend day. To calculate diet composition, this information was verified and entered into the Nutritionist Pro database using their software (Nutritionist Pro; AXXYA Systems, Stafford, TX) by a trained nutrition student. Dietary intake data are presented in **Table 4**.

Serum Measurements

A licensed phlebotomist collected fasted (10 hr) blood samples between 0600 and 0800 hr. Blood samples were allowed to clot for a minimum of 30 minutes. Serum was separated from whole blood by centrifuging for 10 minutes at 4°C @ 2000 × g and in-house aliquots were stored at -80°C until analyses. Serum sVCAM-1, sICAM-1, and sE-selectin were measured (**Table 5**) using human enzyme-linked immunosorbent assay kits (Quantikine Immunoassay; R&D Systems; Inc.; Minneapolis, MN) with the use of a microplate reader (Synergy 2 Multit-mode Microplate Reader; Biotek Instruments Inc.; Winooski, VT). Serum samples were diluted 20-fold for the measurement of sICAM-1 and sVCAM-1 and 10-fold for E-selectin. Inter- and intra-assay coefficients of variation, respectively, were 3.7 and 5.2% for VCAM-1, 3.5 and 3.8% for ICAM-1, and 2.3 and 4.6% for E-selectin assays. Serum samples were also analyzed by a certified clinical laboratory (LabCorp; Kansas City, KS) for a complete metabolic profile to

ensure that participants were otherwise healthy (Table 3). To quantify and predict overall general CVD risk, parameters including age, total cholesterol, HDL cholesterol, SBP, smoking status, and evidence of diabetes were scored using the Framingham Heart Study General CVD 10-Year Risk multivariate risk factor algorithm (D'Agostino et al. 2008).

Statistical Analysis

Statistical analyses were performed using SAS software (Version 9.2; SAS Inc, Cary NC). Results with P values < 0.05 were considered statistically significant. Median (lower, upper quartile) values are presented for urinary analyte excretion (Table 2) and dietary intake (Table 4) data because these data were not normally distributed. To detect differences among the treatment groups at baseline and week 8, urinary isoflavone excretion and urinary sodium and potassium data were analyzed using a nonparametric Kruskal-Wallis test. To determine differences in urinary isoflavone excretion at 8 weeks, we used a follow-up Tukey multiple comparison test. Mean ($\pm 95\%$ confidence interval; minima and maxima) values for data on descriptive characteristics that approximated a normal distribution (age; height; weight; BMI; waist circumference; total cholesterol; LDL cholesterol; HDL cholesterol; triglycerides; serum glucose) are presented in Table 3. To detect differences among treatment groups at baseline, subject characteristics data were analyzed using an analysis of variance (ANOVA). Potential treatment effects on the response variables (serum sVCAM-1, sICAM-1, and sE-selectin) over time (Table 5) were tested using repeated measures ANOVA, with the inclusion of gender as a covariate. Treatment effects were further analyzed with a multivariate analysis of variance (MANOVA), but this analysis did not alter the results (and hence these data are not reported).

RESULTS

Subject Characteristics and Dietary Intake

We enrolled healthy prehypertensive/Stage 1 hypertensive men and premenopausal women in a randomized Iowa Soybean Association-funded clinical study. Among the 144 individuals who completed pre-baseline screening, 73 were eligible to participate in baseline testing, of which 72 met the criteria and were randomly assigned to treatment within the confines of tolerance to soy beverage treatment. A total of 68 participants (60 men and 8 women) completed the eight week trial. Baseline characteristics of all participants according to treatment are presented in Table 3. There were no statistically significant differences among treatment groups at baseline for these variables. Although participants ranged in age from 18 to 63 y, the majority were young adults, ages 26 to 32 y. Nearly all

participants were non-smokers, with the exception of one daily smoker in each treatment group. Overall, each treatment group was considered overweight based upon their mean BMI, whereas the study sample included 23 normal weight (BMI 18.5-24.9), 28 overweight (BMI 25.0-29.9), and 17 class I obese participants (BMI 30.0-34.9). Although the majority of participants had waist circumference measurements considered “normal-risk”, one female and eleven male participants exceeded the cutoff point (>88 cm for women, >102 cm for men) and were considered “high risk”. Resting and mean 24-hour blood pressure for the entire sample fell within the prehypertensive ranges, including those 45 considered prehypertensive and 23 considered Stage I hypertensive. For each treatment beverage group, the mean value fell within the normal range for serum total cholesterol (<200 mg/dL), HDL cholesterol (>40 mg/dL), triglycerides (<150 mg/dL), and fasting glucose (<100 mg/dL); however, mean LDL cholesterol in the SPI beverage group (114 mg/dL) was slightly above the “optimal” cut-off (<100 mg/dL). We observed SBP > 160 mmHg for one male participant who provided written permission from his physician to continue participation. We observed high total cholesterol for one male (289 mg/dL) and one female (298 mg/dL) participant who provided written permission from their physicians to continue participation. When parameters were scored to predict 10-year CVD risk, participants overall exemplified 4.3% risk of CVD at baseline (D’Agostino et al. 2008).

Dietary intake data are presented in Table 4. Carbohydrate, protein, and fat consumption fell within recommended macronutrient distribution ranges for each treatment group. In comparison to established Dietary Reference Intakes (DRIs), participants consistently consumed excess sodium, whereas intakes of magnesium, potassium, copper, and zinc were below the DRIs. Vitamins A, D, and K were also below the DRIs across treatment groups, in addition to biotin and pantothenic acid.

Urinary Analyte Excretion and Compliance

Urinary electrolyte and total urinary isoflavone excretion for each treatment group are presented in Table 2. Urinary sodium and potassium excretion were not significantly different among treatment groups at baseline or week 8. Urinary isoflavone excretion was higher in the soy beverage groups compared with the control group, thus reflecting adherence to treatment. Based upon the nonparametric Kruskal-Wallis test, we found significant differences in urinary isoflavone excretion among the three treatment groups at week 8, as well as between each treatment comparison (based upon follow-up Tukey multiple comparison test). Compliance indicated by leftover milk returned in half gallon milk cartons and subjective report reflected excellent treatment adherence, with 98% of

participants at least 90% compliant. However, these self reports are subjective and thus susceptible to participant reporting error.

Serum Cell Adhesion Molecules

Median (lower, upper quartile) serum concentrations of ICAM-1, VCAM-1, and E-selectin at baseline and week 8 are presented for each treatment group in Table 5. CAM concentrations were not consistently related to blood pressure, with the exception that E-selectin was significantly positively correlated to mean 24-hour SBP and DBP at baseline. ANOVA indicated that there was no time by treatment interaction (no treatment effect) or age effect, but there was a gender effect for ICAM-1 ($P=0.0037$), whereas gender did not reach significance for E-selectin ($P=0.067$) or VCAM-1 ($P=0.16$). Given these results, gender was important to consider as a potentially confounding factor in the analysis, whereas removing women from the analysis (focusing on men only) did not alter the overall results. Men had higher circulating concentrations of ICAM-1 and E-selectin, respectively, at both baseline ($P = 0.0071$ and $P = 0.049$) and week 8 ($P = 0.0054$ and $P = 0.038$).

DISCUSSION

Daily intake of cow's milk or soy beverages prepared from WSB or SPI for eight weeks did not significantly alter soluble CAM concentrations in prehypertensive/Stage I hypertensive individuals. Because CAM serve as biomarkers of atherosclerotic CVD, a reduction in CAM concentration in high-risk individuals, such as hypertensives, may reduce the risk of CVD and a dietary treatment may offer an alternative preventive measure in otherwise healthy early stage hypertensive individuals. Although previous clinical studies have examined the effect of dairy and soy product intake on CAM, this study was unique in that we included premenopausal women and men, whereas prior investigations examined postmenopausal women almost exclusively. Nonetheless, our results are in general agreement with previous findings.

Our results were similar to those of Blum et al. (2003) who provided hypercholesterolemic postmenopausal women with 25 g of soy protein and protein placebo daily for 6 weeks using a crossover design, with a 1 month washout period. The soy protein did not affect sVCAM-1, sICAM-1, or sE-selectin in comparison with the placebo (Blum et al. 2003). Steinberg et al. (2003) enrolled postmenopausal women who consumed 25 g/d of 2 soy products (SPI with isoflavones [107 mg/d]; SPI with trace isoflavones [2 mg/d]); and total milk protein (no isoflavones) for 6 weeks each, with 4 week washout periods between treatments. No significant changes in CAM were observed among the three

dietary groups. In a similar study (Hall et al. 2005), postmenopausal women consumed isoflavone-enriched (50 mg/d) or placebo cereal bars daily for eight weeks, with no change in CAM concentrations. However, one interesting note from these authors indicated that women in an estrogen receptor- β gene polymorphic subgroup exhibited some improvement in VCAM-1 ($P < 0.05$). Finally, postmenopausal women with a history of breast cancer were given isoflavone (114 mg) or placebo tablets daily for 3 months with no treatment difference reported in sE-selectin concentration (Nikander et al. 2003). Although no significant change in E-selectin occurred, serum daidzein ($r = 0.39$; $P < 0.003$) and genistein ($r = 0.38$; $P < 0.004$) concentrations were inversely associated with E-selectin concentration (Nikander et al. 2003).

Not all results are consistent with these reports or the findings of our study. Men and postmenopausal who were provided isoflavone tablets (80 mg/d total isoflavones) for six weeks experienced a significant reduction in sVCAM-1 ($P = 0.009$) (Teede et al. 2003). Nasca et al. (2008) supplemented healthy postmenopausal women with a therapeutic lifestyle change diet alone or a therapeutic lifestyles change diet which replaced 25 g of nonsoy protein with 0.5 cups of soy nuts (25 g soy protein, 101 mg total isoflavones) for 8 weeks. The women who consumed this diet daily did not experience significant reductions in sVCAM-1 or sICAM-1, with the exception of a reduction in sVCAM-1 for hypertensive versus normotensive women who consumed the soy protein rich lifestyle change diet (Nasca et al. (2008). Whereas previous studies found significant positive correlations between SBP and sICAM-1 ($r = 0.50$, $P = 0.02$) (Chae et al. 2001), DBP and sICAM-1 ($r = 0.49$, $P = 0.03$), and SBP and sVCAM-1 ($r = 0.43$, $P = 0.05$) (DeSouza et al. 1997), our study did not replicate these results. E-selectin was significantly and positively correlated to baseline mean 24-hour SBP ($r = 0.30$, $P = 0.013$) and DBP ($r = 0.30$, $P = 0.014$), but not to in-house 10-minute resting blood pressure or to any blood pressure measure at week 8.

In our study, treatment groups were provided two levels of dietary isoflavones (SPI: 30.1 mg/d; WSB: 91.4 mg/d) compared with no isoflavones from cow's milk. The greatest amount of isoflavones consumed daily (WSB: 91.4 mg/d) was similar to amounts previously reported whereby beneficial effects were noted on CAM and other clinical risk factors of atherosclerotic CVD, such as elevated serum cholesterol (Zhan and Ho 2005) and blood pressure (He et al. 2005; Jenkins et al. 2002). Thus, lack of treatment effect in our study was not likely due to the isoflavone content of the beverages.

The presence of soluble CAM in human sera has been implicated in atherosclerotic CVD, as CAM have been found locally in atherosclerotic plaques (Davies et al. 1993; O'Brien et al. 1996) and were

higher in circulation for individuals with atherosclerosis compared with healthy individuals (Hwang et al. 1997; Peter et al. 1997). As markers of atherosclerosis, the biological relevance of the CAM concentrations observed in our participants (Table 5) may suggest that they had near normal risk of atherosclerotic CVD, although our participants had above normal blood pressure. In comparison to mean CAM concentrations in patients with incident coronary heart disease (CHD) (sICAM-1 = 288.7 ng/mL; sE-selectin = 38.2 ng/mL) or coronary artery atherosclerosis (CAA) (sICAM-1 = 283.6 ng/mL; sE-selectin = 41.5 ng/mL), our participants had CAM concentrations (Table 5) at or below the values observed for healthy controls or the reference group (sICAM-1 = 244.2 ng/mL; sE-selectin = 32.8 ng/mL) (Hwang et al. 1997). Note that the median (lower, upper quartile) values presented for our data (they were not normally distributed) may not be entirely comparable with published mean values. Because the mean values for each marker were slightly higher than the corresponding median values in our participants, this suggested that our mean values were comparable to published mean values. Based upon these findings, our participants were likely at greater risk of atherosclerotic CVD primarily because of their prehypertensive/hypertensive state. However, we cannot suggest from our results that the CAM concentrations we observed placed our participants at overall high risk of CVD. Further, although mean Framingham 10-year CVD risk score was 4.3%, in comparison to Framingham risk scores standardized for age and gender (Wilson et al. 1998), our participants on average exhibited risk accounting for only 90% of their expected average risk. To standardize these risk scores, each score was divided by the average expected score for that particular individual based on age and gender. This low risk is attributed to our sample primarily being relatively young adults and overall lacking in other risk factors, such as diabetes or hypercholesterolemia. Our participants were individuals with early stage HTN who were otherwise healthy.

Our inability to demonstrate a treatment effect on CAM was initially suspected to be explained by baseline CAM concentrations that were similar to normotensive individuals and hence within the expected range for healthy individuals. In comparison to mean CAM concentrations (ng/mL) reported by DeSouza et al. (1997) for hypertensive (sICAM-1 = 232.4 ± 16.5 ; sVCAM-1 = 737.3 ± 65.6) and normotensive (sICAM-1 = 189.8 ± 11.2 ; sVCAM-1 = $565.7 \pm 4/6.8$) individuals, concentrations observed in our participants (Table 5) were comparable. The majority of our hypertensive and prehypertensive individuals were young, otherwise healthy adults, which may account for the lower CAM concentrations compared to reported values for hypertensive individuals in other studies. Further, when examined separately at baseline, no significant differences in CAM concentrations were noted between

hypertensive and prehypertensive participants. It is important to note that although participants in the present study had elevated blood pressure, mean blood pressure for this group (resting or mean 24-hour) fell within the prehypertensive range. Whereas baseline CAM concentrations appeared similar to normotensive individuals, previous reports have indicated a significant reduction in CAM with isoflavone supplementation despite baseline concentrations being much lower (Atteritano et al. 2007) than those observed in our participants. Also, studies that reported significant reductions typically enrolled three to four times the number of participants in comparison to the present study. Therefore, it may not be appropriate to attribute the lack of treatment effect to relatively low baseline CAM concentrations, but rather to the relatively small sample size given the large variability in these markers.

Despite being prehypertensive, our participant CAM concentrations reflected that of normotensive individuals, raising speculation as to whether a blood pressure threshold must be reached to raise CAM concentrations. Such a threshold has yet to be identified. We also found no correlation between blood pressure and CAM, with the exception of E-selectin and baseline 24-hour blood pressure measures. Further work in this area, examining a wider range of blood pressures, would be necessary to identify a blood pressure threshold above which CAM concentrations are elevated. Likewise, clinical reference ranges indicative of low or high CAM concentrations do not yet exist; these values would be very useful in the interpretation of results. Although significant time effects were observed for sE-selectin and sVCAM-1 (+4.2% and -8.5%, respectively), these changes may not be large enough to be biologically important. Also, if these markers were truly indicative of 'normal' risk at baseline, a reduction in any marker would not necessarily lessen the risk. The positive change in sE-selectin observed at week 8, albeit significant, was an increase of only 1.6 ng/mL, and did not reach the concentration observed in patients with CVD. Considering that the CAM concentrations in our participants likely indicated 'normal' risk, we should not expect a similar magnitude of response to treatment with individuals who have higher baseline CAM concentrations. It is also important to mention the great inter- and intra-individual variability in these biomarkers, contributing to difficulty in demonstrating statistical significance. These points illustrate that one must have available clinical reference ranges to interpret the biological importance of changes in these CAM biomarkers.

The present study is limited in that participants were enrolled based upon blood pressure inclusion criteria, without knowledge of their CAM concentrations prior to baseline. Because our participants did not exhibit elevated CAM concentrations at baseline, this may have restricted our ability to demonstrate a treatment effect or change over time. Also, we did not recruit normotensive

individuals to serve as the control group, thereby limiting our ability to compare CAM concentrations in an at-risk group to those with 'normal' risk. Including normotensive individuals may have provided further insight as to whether our prehypertensive/Stage 1 hypertensive participants should be considered at-risk based upon their CAM values. In addition, enrolling an equal number of Stage I hypertensive versus prehypertensive participants may have more clearly demonstrated the relationship between CAM concentrations and blood pressure. However, we enrolled 23 hypertensive versus 45 prehypertensive participants in the present study. It may be possible that we included participants who were not truly hypertensive throughout the study because we based their classification on prescreening blood pressure measures that may have been higher than their typical or subsequent blood pressure. Hence, the mean blood pressure for our participants reflected mainly the prehypertensive category, perhaps partially accounting for the undetectable treatment effect. In addition to these limitations, our sample size was relatively small given the large variability in these markers. The Framingham risk score calculations are primarily designed for individuals who are ages 30-74, however participants in this study ranged from ages 18-63. These calculations require age 30 to be used in place of any age less than 30, thus these results may not accurately reflect CVD risk for individuals less than 30 years of age.

Our study design did not include a true control group. Although we did not expect the same magnitude of response from the cow's milk group, all participants received a treatment beverage. Thus, we were unable to compare change in CAM concentrations to individuals without any treatment, which is exceedingly difficult in dietary intervention studies. In addition, we did not incorporate a washout period before participants began receiving treatment. Although the soy food intake questionnaire and baseline urinary isoflavone excretion data indicated that the vast majority of the participants did not consume appreciable amounts of soy products, there was a range of soy intake prior to starting treatment. It is possible, although not probable, that this may have contributed to the lack of treatment effect. Finally, the enrollment of a far greater number of men ($n=60$) than women ($n=8$) made interpretation of a gender effect difficult, and because previous research in this area included primarily postmenopausal women, we did not have comparable values for men or premenopausal women from the literature. It proved exceedingly difficult to recruit premenopausal women who were hypertensive who also had a BMI < 35. Nevertheless, we demonstrated that gender was an important factor to consider in the analysis.

Despite these noted limitations, the strength of this study was the inclusion of healthy individuals with isolated prehypertension or Stage 1 hypertension at relatively low risk of CVD. Hence,

one interpretation might be that dietary intervention does not measurably improve CAM in early stage hypertensive but otherwise healthy individuals. Perhaps decreasing CAM concentrations may not be the mechanism by which soy protein with isoflavones might reduce the risk of CVD in early stage hypertensive individuals. Perhaps isoflavone treatment may be more beneficial to individuals exhibiting a myriad of CVD risk factors, but our study was not designed to test this hypothesis. Our study was also unique in that it included premenopausal women, thus making a contribution in CAM values to the literature. It is well established that postmenopausal women are at increased risk of CVD compared to their premenopausal counterparts. However, the progression of atherosclerosis occurs over the entire life span. Thus, ameliorating the progression of atherosclerosis in younger years should favorably impact atherosclerotic CVD risk later in life. Enrolling prehypertensives, in addition to early stage hypertensives, was intended to allow the comparison of CAM concentrations between these blood pressure groups. However, for reasons provided above, we did not find significant differences between the prehypertensive and hypertensive participants.

In conclusion, prehypertensive/Stage I hypertensive individuals who consumed either cow's milk or soy beverages (prepared from WSB or SPI) for 8 weeks daily did not show any change in soluble CAM concentrations. Consequently, we cannot suggest that daily intake of either cow's milk or soy protein beverages improves circulating CAM concentrations and hence risk of atherosclerotic CVD in similar individuals. Further, as had been reported in some groups of hypertensive individuals, our data suggested that CAM concentrations were not elevated at baseline in potentially at-risk prehypertensive individuals.

ACKNOWLEDGEMENTS

We would like to thank the participants who diligently adhered to the dietary intervention and reported faithfully to our research unit regularly. We would like to thank the dietetics students (particularly Abby Pollard, Heather (Meardon) Plizga, and Tiffany McCabe) in the Department of Food Science and Human Nutrition at Iowa State University who helped with lab processing, data collection, dietary intake analysis, and data entry. We would like to thank Dennis Lock, a doctoral student in the Department of Statistics at Iowa State University, for his efforts with data analysis.

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TABLES**Table 1.** Nutrient composition of treatment beverages

	Cow ^a	SPI ^b	WSB ^c
	240 mL (1 cup)	240 mL (1 cup)	240 mL (1 cup)
Serving size	240 mL (1 cup)	240 mL (1 cup)	240 mL (1 cup)
Energy (kcal)	100	100	100
Protein (g)	8	6	6.25
Total Fat (g)	2.5	3	3.5
Saturated fat (g)	1.5	0.5	0.5
Trans fat (g)	0	0	0
Polyunsaturated fat (g)	-	-	2
Monounsaturated fat (g)	-	-	1
Cholesterol (mg)	15	0	0
Total Carbohydrates (g)	13	11	10
Fiber (g)	0	0	1
Sugars (g)	13	10	7
Sodium (mg)	125	105	95
Potassium (mg)	410	-	300
Phosphorus (mg)	-	-	-
Magnesium (mg)	-	-	40
Zinc (mg)	-	2.25	1.5
Calcium (mg)	300	300	300
Iron (mg)	-	1.1	1.1
Selenium (µg)	-	-	5.6
Vitamin A (IU)	500	500	500
Vitamin C (mg)	2.4	-	-
Vitamin D (IU)	100	100	120
Riboflavin (mg)	-	-	0.5
Folate (µg)	-	-	24
Vitamin B12 (µg)	-	1.5	3
Total Isoflavones (mg)	-	8.6	26.1
Genistein (mg)	-	5.1	16.1
Daidzein (mg)	-	2.3	8.8
Glycitein (mg)	-	1.2	1.2

^a Cow: Cow's milk beverage^b SPI: Soy beverage prepared from soy protein isolate^c WSB: Soy beverage prepared from whole soy bean

Table 2. Urinary analyte excretion

	Treatment Beverage Group							P value
	Cow ^a (n= 21)		SPI ^b (n= 23)		WSB ^c (n= 24)			
Total Urinary Isoflavone (μmol/L)	Median (lower, upper quartile values)							
Baseline	0.0	(0.0, 0.0)	0.0	(0.0, 0.0)	0.0	(0.0, 0.0)	0.53	
Week 8	0.5	(0.0, 2.4)	6.7	(3.7, 11.7)	17.6	(7.1, 28.0)	<0.001 ^d	
Urinary sodium (mmol/day)								
Baseline	197	(156, 235)	193	(142, 243)	180	(148, 211)	0.75	
Week 8	135	(104, 167)	131	(91, 190)	146	(109, 191)	0.61	
Urinary potassium								
Baseline	65	(47, 84)	67	(49, 84)	60	(50, 69)	0.98	
Week 8	54	(45, 80)	45	(28, 73)	69	(47, 90)	0.12	

Median (lower, upper quartile values)

^a Cow: Cow's milk beverage

^b SPI: Soy beverage prepared from soy protein isolate

^c WSB: Soy beverage prepared from whole soy bean

^d Significant differences among treatment beverage groups using Kruskal-Wallis ($P < 0.05$)

Table 3. Characteristics of participants at baseline

Characteristic	Treatment Beverage Group			
	Cow ^a (n= 21)	SPI ^b (n= 23)	WSB ^c (n= 24)	All (N = 68)
		Mean (95% CI) (min,max)		Mean (95% CI) (min,max)
Age (years)	30 (26, 32) (19, 63)	29 (24, 34) (19, 63)	29 (24, 34) (18, 53)	29 (26, 32) (18, 63)
Gender (n [%])				
Male	21 (100%)	20 (87%)	19 (79%)	60 (88%)
Female	0	3	5	8
Race/Ethnicity (n [%])				
White	18 (85.7%)	16 (69.6%)	19 (79.2%)	53 (77.9)%
Asian	1 (4.8%)	6 (26.1%)	4 (16.7%)	11 (16.2%)
Black	1 (4.8%)	0	1 (4.2%)	2 (2.9%)
Hispanic	1 (4.8%)	1 (4.3%)	0	2 (2.9%)
Height (cm)	179.4 (173.8, 184.9) (132.3, 193.9)	175.7 (170.8, 180.7) (147.5, 197.2)	178.6 (172.2, 182.9) (149.4, 193.9)	177.8 (175.1, 180.6) (132.3, 197.2)
Males	179.4 (173.8, 184.9)	179.6 (175.8, 183.4)	181.3 (178.2, 184.4)	180.1 (177.6, 182.6)
Females	- -	161.8 (153.5, 170.1)	159.4 (151.2, 167.7)	160.9 (154.4, 167.4)
Weight (kg)	88.7 (81.4, 96.1) (63.9, 122.8)	84.5 (77.2, 91.9) (57.8, 128.2)	88 (81.5, 94.5) (56.6, 115.5)	87 (83.2, 90.9) (56.6, 128.2)
Males	88.7 (81.8, 95.6)	88.6 (81.4, 95.9)	91.6 (86.1, 97.1)	89.7 (85.9, 93.4)
Females	- -	69.7 (59.1, 80.4)	63.1 (57.5, 68.9)	67.3 (59.4, 75.1)
BMI (kg/m ²)	26.7 (24.6, 28.7) (20, 36)	27.1 (25.2, 29.0) (21, 35)	27.3 (25.8, 28.7) (21, 32)	27.0 (26.0, 28.0) (20, 36)

Table 3. (Continued)

Characteristic	Cow ^a (n= 21)		SPI ^b (n= 23)		WSB ^c (n= 24)		All (N = 68)	
Waist circumference (cm)	91.4	(84.5, 98.4)	89.7	(84.5, 95.0)	91.5	(86.6, 96.3)	90.9	(87.8, 94.0)
		(72.5, 126.0)		(72.5, 115.0)		(72.6, 117.1)		(72.5, 126.0)
Males	91.4	(84.5, 98.4)	92.6	(89.3, 98.2)	93.7	(87.2, 98.0)	92.6	(89.5, 95.7)
Females	-	-	75.7	(70.0, 81.3)	79.3	(69.2, 86.5)	78.0	(78.0, 82.8)
Smoking status (n)								
Non-smoker		17		22		23		26
Recently quit		1		0		0		1
1-2 times/month		1		0		0		1
1-2 times/week		1		0		0		1
Daily		1		1		1		3
Resting BP (mmHg)								
SBP	132	(129, 135)	133	(130, 136)	136	(132, 140)	134	(132, 136)
		(120, 143)		(120, 143)		(118, 155)		(118, 155)
DBP	79	(75, 84)	84	(81, 88)	86	(81, 90)	83	(81, 86)
		(63, 100)		(70, 98)		(67, 123)		(63, 123)
Mean 24 hr ambulatory BP (mmHg)								
SBP	136	(131, 140)	134	(129, 138)	136	(133, 140)	135	(133, 138)
		(120, 157)		(118, 167)		(117, 151)		(117, 167)
DBP	77	(73, 81)	78	(74, 81)	78	(74, 81)	78	(73, 81)
		(67, 99)		(68, 100)		(63, 94)		(67, 100)
Serum total cholesterol (mg/dL) ^d	168	(154, 182)	185	(167, 203)	172	(156, 188)	175	(166, 184)
		(133, 249)		(130, 289)		(119, 298)		(119, 298)
Serum LDL (mg/dL) ^d	98	(85, 111)	114	(100, 128)	100	(86, 113)	104	(96, 112)

Table 3. (Continued)

Characteristic	Cow ^a (n= 21)	SPI ^b (n= 23)	WSB ^c (n= 24)	All (N = 68)
	(56, 169)	(65, 177)	(60, 214)	(56, 214)
Serum HDL (mg/dL) ^d	45 (41, 49) (31, 58)	47 (42, 51) (29, 73)	44 (40, 48) (28, 73)	45 (43, 48) (28, 73)
Serum triglycerides (mg/dL) ^d	127 (94, 159) (43, 282)	124 (89, 159) (44, 328)	140 (110, 170) (49, 285)	130 (112, 149) (43, 328)
Fasting glucose (mg/dL) ^d	85 (82, 89) (76, 113)	89 (84, 93) (73, 121)	88 (85, 91) (76, 111)	87.4 (85, 89) (73, 121)
Metabolic syndrome score ^d	1.4 (0.9, 1.9) (0, 3)	1.5 (1.1, 2.0) (0, 3)	1.6 (1.2, 2.0) (0, 3)	1.5 (1.3, 1.7) (0, 3)
Urinary sodium (mmol/d)	197 (156, 235) (85, 430)	193 (142, 243) (25, 498)	180 (148, 211) (75, 359)	189 (167, 212) (25, 498)
Urinary potassium (mmol/d)	65 (47, 84) (22, 215)	67 (49, 84) (23, 191)	60 (50, 69) (21, 104)	64 (56, 72) (21, 215)

Mean (95% CI) (min,max)

BP: Blood pressure; LDL: Low-density lipoprotein; HDL: High-density lipoprotein

^a Cow: Cow's milk beverage

^b SPI: Soy beverage prepared from soy protein isolate

^c WSB: Soy beverage prepared from whole soy bean

^d One data point missing from SPI treatment beverage group

^e Metabolic syndrom score based on American Heart Association/Updated NCEP guidelines: waist circumference: men \geq 102 cm, women \geq 88 cm; triglycerides \geq 150; HDL men $<$ 40, women $<$ 50; SBP \geq 130 and/or DBP \geq 85; fasting glucose \geq 100

Table 4. Energy and nutrient intake at baseline

Nutrient	Treatment Beverage Group							
	Cow ^a (n= 21)		SPI ^b (n= 23)		WSB ^c (n= 24)		All (N = 68)	
	Median (lower, upper quantile values)							
Energy (kcal)	2675	(2362, 3038)	2300	(1887, 2898)	2869	(2286, 3121)	2614	(2106, 3042)
Protein (g)	92.4	(74.7, 128.0)	91.7	(71.9, 138.9)	102.9	(80.1, 135.4)	95.3	(75.3, 133.2)
Total Fat (g)	96.3	(77.0, 105.7)	85.2	(51.6, 119.7)	97.0	(78.6, 119.6)	92.1	(66.4, 115.7)
Saturated (g)	32.5	(20.5, 40.2)	25.3	(16.8, 44.1)	31.0	(26.8, 42.3)	30.9	(21.9, 41.2)
Monounsaturated (g)	23.8	(20.4, 32.3)	19.8	(13.5, 35.5)	22.6	(16.7, 34.9)	23.0	(16.7, 33.5)
Polyunsaturated (g)	12.7	(8.1, 15.8)	10.6	(7.0, 16.4)	11.0	(7.1, 16.9)	11.7	(7.2, 16.5)
Trans (g)	0.5	(0.1, 1.9)	0.4	(0.0, 1.3)	1.0	(0.4, 2.1)	0.7	(0.1, 1.8)
Carbohydrates (g)	315	(259, 388)	282	(200, 311)	322	(286, 357)	311	(246, 370)
Dietary Fiber (g)	16.3	(11.7, 23.5)	17.7	(12.0, 26.1)	16.3	(11.5, 27.5)	17.0	(11.7, 24.8)
Calcium (mg)	1010	(811, 1566)	885	(523, 1366)	1198	(879, 1529)	1028	(672, 1495)
Phosphorus (mg)	1245	(853, 1593)	1092	(665, 1979)	1247	(1027,1793)	1230	(848, 1700)
Magnesium (mg)	252	(220, 338)	189	(117, 389)	273	(204, 383)	250	(160, 360)
Sodium (mg)	3646	(3417, 4810)	3764	(2593, 4939)	4225	(3570, 5073)	3930	(2986, 4914)
Potassium (mg)	2642	(1910, 3068)	1914	(1432, 3637)	2526	(1658, 3399)	1628	(2377, 3308)
Iron (mg)	17.2	(13.2, 26.5)	16.8	(9.6, 27.1)	21.3	(13.9, 28.7)	18.2	(12.0, 27.3)
Zinc (mg)	13.7	(11.6, 18.8)	8.7	(5.4, 20.4)	12.7	(8.4, 22.3)	13.3	(8.1, 2.1)

Table 4. (Continued)

Nutrient	Cow ^a (n= 21)		SPI ^b (n= 23)		WSB ^c (n= 24)		All (N = 68)	
Copper (mg)	1.2	(0.9, 1.5)	1.1	(0.5, 1.9)	1.3	(0.9, 2.0)	1.2	(0.8, 2.0)
Vitamin A (IU)	4477	(2925, 7121)	5564	(2199, 8870)	6390	(3760, 9152)	5038	(2515, 8208)
Vitamin D (IU)	229	(113, 395)	192	(61, 667)	108	(15, 418)	165	(5, 426)
Vitamin E (IU)	3.2	(1.3, 12.4)	3.4	(0.1, 32.0)	2.9	(0.8, 32.7)	3.3	(0.6, 31.3)
Vitamin K (µg)	31	(21, 53)	44	(25, 110)	50	(32, 80)	40	(23, 75)
Vitamin C (mg)	58	(41, 120)	94	(47, 168)	102	(43, 180)	94	(42, 153)
Vitamin B1 (mg)	1.8	(1.6, 2.2)	1.9	(1.0, 3.0)	1.6	(1.2, 3.1)	1.9	(1.1, 3.0)
Vitamin B2 (mg)	2.5	(2.1, 2.8)	2.2	(1.2, 3.7)	2.3	(1.5, 4.2)	2.4	(1.5, 3.7)
Vitamin B3 (mg)	26.2	(19.6, 33.4)	27.3	(13.8, 47.3)	26.3	(18.4, 45.5)	26.4	(16.9, 40.7)
Pantothenic acid (mg)	5.3	(4.2, 7.1)	4.6	(2.9, 12.2)	4.9	(2.8, 12.7)	4.9	(2.9, 11.5)
Vitamin B6 (mg)	2.1	(1.4, 2.4)	1.9	(0.8, 4.5)	2.1	(1.2, 4.3)	2.1	(1.1, 3.5)
Biotin (µg)	18.3	(12.6, 27.1)	20.0	(5.7, 40.5)	16.1	(4.3, 47.5)	19.1	(6.4, 38.5)
Vitamin B12 (µg)	6.6	(4.8, 9.6)	6.3	(2.9, 9.4)	7.4	(3.1, 13.4)	6.5	(3.6, 12.0)
Alcohol (g)	27.3	(0.0, 47.4)	0.0	(0.0, 3.0)	5.6	(0.0, 33.5)	2.5	(0.0, 36.5)

Median (lower, upper quantile values)

Determined from 3-day food diaries recorded at baseline

^a Cow: Cow's milk beverage

^b SPI: Soy beverage prepared from soy protein isolate

^c WSB: Soy beverage prepared from whole soy bean

Table 5. Soluble endothelial adhesion molecules at baseline and after 8 weeks of treatment beverage intake

	Treatment Beverage Group						ANOVA (<i>P</i> value)	
	Cow ^a (<i>n</i> = 21)		SPI ^b (<i>n</i> = 23)		WSB ^c (<i>n</i> = 24)		Time*Treatment	Gender
I-CAM-1 (ng/mL)								
Baseline	214.8	(135.5, 268.9)	184.5	(151.0, 223.4)	179.7	(142.8, 214.7)	0.32	0.0037
Week 8	192.8	(141.4, 239.9)	201.4	(148.6, 231.2)	187.9	(131.2, 233.1)		
Change	-10%		9%		5%			
VCAM-1 (ng/mL)								
Baseline	480.3	(431.1, 625.9)	488.8	(326.4, 560.8)	506.7	(443.9, 554.4)	0.85	0.16
Week 8	463.5	(423.0, 535.7)	389.9	(352.7, 481.8)	474.7	(347.9, 589.1)		
Change	-3%		-20%		-6%			
E-selectin (ng/mL)								
Baseline	37.2	(32.3, 50.0)	36.4	(29.2, 49.8)	38.1	(28.0, 47.6)	0.23	0.067
Week 8	37.4	(31.5, 52.2)	36.8	(31.0, 47.2)	43.7	(32.2, 54.6)		
Change	1%		1%		14%			

Median (lower, upper quartile values)

^a Cow: Cow's milk beverage

^b SPI: Soy beverage prepared from soy protein isolate

^c WSB: Soy beverage prepared from whole soy bean

I-CAM-1: Intercellular adhesion molecule 1; VCAM-1: Vascular cell adhesion molecule 1; E-selectin: Endothelial leukocyte adhesion molecule 1

ANOVA indicated no time*treatment interaction, but gender effect for I-CAM-1

GENERAL CONCLUSIONS

In summary, results for the primary objective of this study indicated that daily consumption of cow's milk or soy protein beverage prepared from SPI or WSB did not alter sCAM concentrations in prehypertensive/Stage I hypertensive men and premenopausal women. Consequently, we cannot suggest that daily intake of either cow's milk or soy protein beverages will improve circulating sCAM concentrations and hence lessen the risk of atherosclerotic CVD in premenopausal women or men. Further, these data suggested that sCAM at baseline in prehypertensive individuals were not elevated to potentially at-risk concentrations, as has been reported in hypertensive individuals. Results pertaining to our secondary objective suggested a gender difference for ICAM-1 and E-selectin (statistical trend) in prehypertensive individuals, demonstrated by significantly greater concentrations observed for men at baseline and week 8.

ACKNOWLEDGMENTS

I would first like to thank my major professor, Dr. D. Lee Alekel. Her guidance throughout my graduate career has provided me an exceptional learning experience. Her mentoring, patience, and encouragement have fostered my development as a graduate student and research assistant. I continue to appreciate her confidence in my work, as well as upholding my efforts to the highest of expectations. As an advisor and instructor, her support has facilitated my learning and will contribute to my future success. Secondly, I am grateful for the expertise of my other committee members, Dr. Warren Franke and Dr. Alicia Carriquiry, and for their contribution to the research project and the development of my thesis. I greatly appreciate their time and assistance.

I would like to recognize Jeanne Stewart for the numerous hours she provided in the lab and her guidance with laboratory analysis. Her patience and attention to detail prepared me with proficient lab techniques. I am also appreciative of the continued support and leadership of Joanne Lasrado-Hollis, who has been a valuable mentor throughout my graduate career. Finally, I would like to thank Dennis Lock for his efforts in statistical analysis for this thesis, and the undergraduate students who contributed to lab processing, data collection, dietary analysis, and data entry.