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Herpes Virus Infections, Inflammatory Markers and Risk of Developing T2DM and CVD: An Analysis of NHANES with Adults, Aged 20-49, 1999-2010

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Margie Irizarry De La Cruz

MS, University of Illinois, 2006

BS, University of Georgia, 1999

Dissertation Submitted in Fulfillment

of the Requirements for the Degree of

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Abstract

Herpes simplex viruses (HSVs), are among the most virulent and widespread pathogens; they affect 60–90% of the population worldwide. Substantial evidence indicates a possible association between pathogens and chronic disease. HSVs, among other viruses, have been associated with increased risk for inflammatory diseases. However, prior findings have been inconsistent on the role of infection in triggering autoimmune response and chronic disease. This study builds on the premise that pathogens can induce an inflammatory response and increase the risk for disease development. A representative U.S. sample from NHANES, a national population-based cross-sectional survey, was used to examine the relationship between HSVs infection and type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD). Results from the two-tailed, Pearson chi-square test and multiple logistic regression analyses found no significant association between HSV or multiple herpes virus infections and T2DM or CVD, which suggest rather a secondary phenomenon. However, all the risk factors examined in this study indicated an association with either T2DM, CVD or both. Two inflammatory markers, C-reactive protein (CRP) and serum ferritin, were significantly associated with T2DM and CVD. These findings have potential implications for social change as they support the premise that high levels of CRP and ferritin may be associated with T2DM and CVD. Existing guidelines for primary and secondary prevention of T2DM and CVD could be expanded (a) to include CRP and ferritin as part of the health assessment for T2DM and CVD in high-risk populations, and (b) to explore the effectiveness of CRP and ferritin as predictive biomarkers and prognostic tools for T2DM and CVD.

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Chapter 1: Introduction to the Study

Background

The growing list of chronic diseases previously considered to have a noninfectious etiology is of great public health concern (SoRelle, 1998). Cancer, for example, one of the most studied chronic diseases, is also recognized as an inflammatory disease that has been linked to an infectious etiology (Schlipköter & Flahault, 2010; Zur Hausen, 2009). One of the mechanisms linked to the comorbidity of pathogens and inflammatory diseases is that infection must be chronically active (Sfriso et al., 2010). This pathogenic mechanism has been observed in infectious agents such as epstein-barr virus (EBV), *Helicobacter pylori* (*H. pylori*), and herpes simplex viruses (HSVs), which are able to elude immune recognition and HSVs can stay dormant and reactivate for the life of the host (Kusters, van Vliet, & Kuipers, 2006; Lima et al., 2008). Of significant note is the fact that infections may be cofactors that indirectly affect genetic mutations, while the direct pathway to disease development may be noninfectious (Christen, Hagen, Shigenaga, & Ames, 1999). This phenomenon can be explained by the fact that a substantial part of the inflammatory disease processes may result from an autoimmune response to chronic inflammation leading to the belief that the etiology of the disease is not related to infection (Christen et al., 1999; Sfriso et al., 2010). Considerable evidence has been accumulated in favor of chronic viral or bacterial infections with cardiovascular diseases, diabetes mellitus, atherosclerosis, and Alzheimer's disease, among other chronic diseases (O'Connor, Taylor, & Hughes, 2006).

Introduction

Researchers are beginning to understand the role that chronic inflammation may play in the pathogenesis of chronic diseases (Lutsey, Pankow, Bertoni, Szklo, & Folsom, 2009). It has been documented that highly virulent agents may induce inflammation as a result of a robust immune response creating a susceptible environment conducive to disease development (Conrady, Drevets, & Carr, 2011; Lutsey et al., 2009; van der Kleij & Yazdanbakhsh, 2003). The biological plausibility of theories linking pathogen-induced inflammation and autoimmune-inflammatory disease has been examined in prior work (Watts, Crimmins, & Gatz, 2008). Although promising findings have been reported, most results have been inconsistent (Bauer & Meyer, 2011; Gómez, Laurés, Baltar, Melón, Díez, & de Oña, 2005; Lutsey et al., 2009; Sethi et al., 2008; Sun et al., 2004).

Over the years, the epidemiology of the highly pathogenic HSVs has changed dramatically. Eight out of the 80 known viruses in the herpes family to date are recognized as human pathogens (Fatahzadeh & Schwartz, 2007). In the United States, trends in genital herpes with HSV type 2 (HSV-2) showed a decrease of 21.0% (95% CI: 19.1-23.1) from 1988-1994 to 17.0% (95% CI; 15.8-18.3) from 1999-2004 among individuals aged 14-49 years (CDC, 2010a, 2010b). Nevertheless, an increase among individuals diagnosed with genital herpes with herpes simplex virus type 1 (HSV-1) has been observed in later years, 1.8% in 1999-2004 versus 0.4% in 1988-1994 ($P<.001$) (Malkin, 2004; Xu et al., 2006; CDC, 2010a, 2010b). This new trend in genital herpes suggests a possible genetic crosstalk or immune-synergy between HSV-1 and HSV-2 infections (Chentoufi et al., 2012; Kimberlin, 2004; Kriebs, 2008; Mark et al., 2008).

Worldwide, the prevalence of HSV is approximately 90% (Malkin, 2004; Smith & Robinson, 2002; Wald & Corey, 2007). In the United States, over 50% of the population is seropositive to HSV1 by the time they reach age 40 and approximately 25% is seropositive to HSV2 (CDC, 2010b; Kimberlin, 2004; Xu et al., 2006). This is of great public health concern given the evidence that people infected with HSV2 are up to three times more likely to acquire HIV and to transmit the HIV virus to others compared to people who are seronegative to HSV-2 and the new synergy trend observed between HSVs and HIV (Chentoufi, 2012; Freeman et al., 2006; Holmberg et al., 1988; McClelland et al., 2002; Schacker et al., 1998). According to the Centers for Disease Control and Prevention (CDC, 2006), transmission risks of HSV-2 are higher since over 80% of those infected with the virus are asymptomatic, and the effectiveness of current prevention measures for HSV-1 and HSV-2 is limited to the use of condoms and prophylactic medications (Leone, 2005; Mell, 2008; Wald et al., 2001) and a potential vaccine still in development (Belshe, 2012; Samandary, 2014). Additionally, the cost of HSV-2 in the U.S. is expected to be nearly $2 billion by the end of 2015 (Szucs, Berger, Fisman, & Harbarth, 2001). To better understand disease risks and to develop effective treatments and prevention strategies, it is imperative to understand the epiphenomenon of infectious agents and their possible correlation with common chronic diseases. Findings from the present study inform the fields of medicine and public health about potential prevention measures for individuals at risk of CVD and/or T2DM, who are also seropositive to herpes virus infection.

Statement of the Problem

HSVs are among the most virulent and widespread pathogens; they affect 60-90% of the population worldwide (CDC, 2010; Smith & Robinson, 2002; Wald & Corey, 2007; Xu et al., 2006). Considering that HSVs have the ability to reactivate during the lifetime of the host, it is possible that the recurrent autoimmune and inflammatory response induced by these viruses may increase the risk for developing inflammatory chronic diseases, including two of the top 10 leading causes of death in the United States: T2DM and CVD (Lloyd-Jones et al., 2010; Lutsey et al., 2009). Most association studies conducted with pathogens and chronic diseases have used small samples of specific populations, including immunocompromised individuals, in clinical settings leading to conflicting results (Rupprecht et al., 2001; Sun et al., 2004). As a result, there is minimal understanding about the intricate relationship between infectious agents and chronic diseases. In this study, I further examined the relationship between human pathogenic HSVs and two chronic diseases, T2DM and CVD.

Purpose of the Study

This study sought to examine the potential relationship between HSV and two chronic diseases, T2DM and CVD in a U.S. representative sample, aged 20-49. Although inferences about causal factors cannot be made with cross-sectional studies, this study was expected to contribute to the existing literature by providing additional evidence on possible association between pathogens and chronic disease. I also examined if a higher burden of pathogens, such as having more than one type of herpes virus—for example, HSV-1, HSV-2, and cytomegalovirus (CMV)—increases the risk for developing T2DM

and/or CVD or if it is merely a secondary phenomenon. Further, I evaluated the relationship between three well-known inflammatory markers (CRP, serum ferritin, and homocysteine) and T2DM and CVD. Lastly, the prevalence of reported HSV infection, T2DM, CVD and selected inflammatory markers were examined to account for a true representative sample.

Research Questions

The following hypotheses derived from the literature and steered the present study:

$H_1$1: Is HSV-1 infection associated with higher risk of developing T2DM?

$H_0$1: HSV-1 infection is not associated with higher risk for T2DM.

$H_1$2: Is HSV-2 infection associated with higher risk for of developing T2DM?

$H_0$2: HSV-2 infection is not associated with higher risk for T2DM.

$H_1$3: Is HSV-1 infection associated with higher risk of developing CVD?

$H_0$3: HSV-1 infection is not associated with higher risk for CVD.

$H_1$4: Is HSV-2 infection associated with higher risk of developing CVD?

$H_0$4: HSV-2 infection is not associated with higher risk for CVD.

$H_1$5: Is having a higher burden of pathogens (HSV-1 + HSV-2+CMV) associated with a higher risk of developing T2DM and/or CVD?

$H_0$5: A higher burden of pathogens is not associated with higher risk for T2DM and/or CVD.

The study methodology including study design, dependent and independent variables, covariates and potential confounders will be discussed in detail in Chapter 3, Methodology.

Definition of Terms

Acute-phase response (APR): The initial response or acute inflammation response of the innate immune system to injurious stimuli caused by harmful agents (Gruys, Toussaint, Niewold, & Koopmans, 2005).

Autoimmune-inflammatory response: A mechanism induced by the innate immune system when tissues are injured by pathogens, trauma, toxins, extreme heat and cold, to name few. Damaged cells release chemicals that in turn cause cells to leak fluid to tissues causing swelling; hence inflammation (Firestein, 2011).

Cardiovascular or heart disease (CVD): According to the World Health Organization (WHO), CVD refers to a variety of disorders of the heart, to include: *Coronary heart disease* (CHD), which is also known as *coronary artery disease* (CAD). CHD is caused by the build-up of plaque on the wall of the heart arteries blocking the blood and oxygen supply to the heart. *Angina* or chest pain is one of the most common risk factors for heart disease. *Heart attack* also known as *myocardial infarction (*MI) is caused by a blood clot, which in turn blocks the blood flow to the heart causing damage to the heart (Bonow RO, Mann, Zipes, & Libby, 2011; Greenland et al., 2012; WHO, 2012).

Type 2 diabetes mellitus: Characterized by an array of metabolic dysfunctions including insulin resistance and inadequate insulin and glucagon secretion. In this study,

the American Diabetes Association (ADA) criteria were utilized for the diagnosis of T2DM. According to Mahler and Adler (1999) "Impaired fasting glucose was defined as fasting plasma glucose of 110 or more and 125 mg/dl or less. Impaired glucose tolerance (IGT) was defined as a 2-h plasma glucose value of 140 or more and of less than 200 mg/dl during an oral glucose tolerance" (p. 1165). Hemoglobin A1C measurements were applied to the diagnoses of diabetes (6.5% or greater) and (5.7%-6.4%) for prediabetes (ADA, 2003; Mahler & Adler, 1999).

Inflammatory markers: Also known as biomarkers are useful clinical diagnostic and prognostic tools for ruling out specific conditions. For example, elevated levels of *C - reactive protein (*CRP), a protein synthesized by the liver, are an indication of inflammation present in the body. As a result, CRP is used as a biomarker for the risk of CVD and T2DM. *Interleukin or IL-6,* a protein-coding gene, functions with inflammation in response to harmful stimuli such as infection. *Fibrinogen*, a protein synthesized by the liver, helps to stop clots from forming by converting thrombin into fibrin and may indicate a risk for diabetes. Elevated levels of *ferritin*, a protein that controls the levels of iron in the blood, may be an indication of inflammation, heart failure, diabetes and other conditions. Elevated levels of *homocysteine,* an amino acid found in the blood plasma, increase the risk for heart disease (Ballantyne & Nambi, 2005; Davidson, 2011; Ridker, Hennekens, Buring, & Rifai, 2000; Watson, Round, & Hamilton, 2012).

Significance of the Study

Social Change Implications

Results from this study are expected to add to the growing body of evidence that links infectious agents with chronic disease. A positive association between HSV and T2DM and CVD would provide the medical community and the public health field with valuable information that can be translated into clinical practice. Serologic testing for HSV could be included as part of a routine health assessment for patients at high risk for T2DM and CVD. Preventive screening could improve the health of susceptible groups, reduce health care cost, and foster policy change. Additionally, existing prevention guidelines for T2DM and CVD could be expanded to include inflammatory biomarkers—including CRP, serum ferritin, and homocysteine—as predictive biomarkers for T2DM and CVD (Oshaug, Bugge, Bjonnes, Borch-Iohnsen, & Neslein, 1995; Braly, & Holford, 2003; Laakso, 2008; Watson et al., 2012). This study could support more collaborative research across disease entities—such as T2DM, CVD and HSV—which are traditionally housed in separate silos for research and funding.

Assumptions, Limitations and Strengths

This study assumed that the survey participants were randomly selected and thus there was little or no selection bias. It is also assumed that the survey instrument used to collect the NHANES data was appropriate to measure the selected variables to test the research questions of this study. The clinical, physical, and self-reported data collected through NHANES annually provides a comprehensive health profile of each survey participant and allows for data validation.

The present study has a few limitations. First, it uses a cross-sectional design, which limits any causal inferences that can be made between associated variables. Second, it evaluates only a select number of inflammatory biomarkers based on those included in the NHANES dataset, rather than a more comprehensive list from a literature review. Third, it is not possible to determine whether the HSV infection included in the analysis is primary or recurring infection with the NHANES data. As a result, inferences cannot be made on truly primary infections versus recurring ones. This limitation also restricts the ability to determine whether any association exists between the chronicity of an HSV infection and the outcomes of interest, T2DM and CVD. Fourth, data to examine the interrelationship between inflammation, HSV and T2DM and/or CVD was not readily available. Fifth, a number of selected independent variables were collected through self-report, which may result in an inaccurate or biased recall.

This study also has several strengths. First, it uses a representative sample from a national population-based survey; hence, conclusions and implications drawn from this study can be applied to the general population. Second, the study uses serological measures for detecting antibodies for HSV-1 and HSV-2, as well as standard indicators for diabetes, CVD and inflammation markers, which allow for drawing conclusions from positive cases. Potential cofounders for T2DM and CVD seem unlikely given that most traditional factors (such as age, gender, smoking, and socioeconomic status) and nontraditional markers (such as C-reactive protein levels) were weighted and controlled for in the study population.

Summary

This is the first study to offer a closer look at the possible association of HSV and two leading chronic diseases, T2DM and CVD, using a nationally representative sample. The study design supports the examination of the cross-sectional relationship between seropositivity of HSV and these two inflammation-prone diseases. Although causal conclusions cannot be made with this cross-sectional study, findings and implications could provide the scientific community with additional evidence on whether a possible association between HSV and T2DM and/or CVD exists. This study is also the first to examine the association between three inflammatory biomarkers and T2DM and CVD, in addition to evaluate the burden of infection among individuals with T2DM or CVD. An overview of each of the independent and dependent variables, study methodology, discussion of study results, and recommendations are discussed in the next chapter.

Chapter 2 begins with a review of the existing literature on the suggested association between infectious diseases and inflammatory response. The chapter includes a description of the pathogenic effect in autoimmune-inflammatory response theory, which serves as the theoretical framework for the current study and provides substance regarding the possible association of innate immunity, inflammation, and pathogen-induced acute response. The chapter also discusses the possible mechanisms involved in the activation of innate immunity of T2DM and CVD. Further, the epidemiology, pathogenesis, and immunopathological aspects of herpes virus infection—as well as the role of inflammation in the pathogenesis of T2DM and CVD—are examined. The chapter

concludes with association studies between HSV and T2DM and/or CVD and research gaps.

Chapter 3 describes the study's methodology, including the analysis conducted to answer the research questions, including the use of regression analyses (chi-square, logistic, and stepwise) as a valid means to measure the possible relationship between HSV and T2DM and/or CVD. In addition, Chapter 3 includes a description of the study design, study population, variables to be studied, potential bias, and ethical considerations.

Chapter 4 offers a summary of the analyses and the results. It describes the demographic and health characteristics of the study population as well as the distribution of the herpes viruses, biomarkers, diabetes and cardiovascular disease in the study sample. The prevalence of T2DM and CVD among individuals with HSV and selected covariates as well as the potential relationship between these are examined. Results are illustrated in tables and figures.

Chapter 5 provides an overview of the findings, the conclusion, and the implications for social change. This chapter also evaluates potential reasons for study's findings and existing evidence in support of them. Since a national representative sample was used in this study, association inferences would be appropriate. Limitations of the present study are also discussed.

Chapter 2: Literature Review

Introduction

A comprehensive review of the literature relevant to this study was conducted. Two electronic databases were searched: Medline and PubMed. Google Scholar and references cited in key articles were also evaluated. Search terms were selected from the literature review from peer-reviewed journals dating back 1970: herpes (inflamed, inflammation or inflammatory) and (disease or diseases or illness), herpes or HSV and (cardiovascular disease or CHD or CVD) and (diabetes, type 2 diabetes mellitus, T2DM) and (inflammation or inflammatory) and (disease or diseases), herpes or HSV and (CHD or CVD) and (inflammation or inflammatory) and (disease or diseases), and T2DM and CVD inflammatory markers. Peer-reviewed articles and/or abstracts in English were retrieved. This chapter provides (a) an overview of the theoretical constructs; (b) the epiphenomena of the autoimmune-inflammatory response, HSV, CHD, and T2DM; (c) the possible interaction among these constructs; and (d) the different mechanisms that could account for an association.

Theoretical Constructs

This study builds on the premise that pathogens, particularly those that can reactivate during the lifetime of the host, can induce an autoimmune-inflammatory response. Chronic, inflammation-prone diseases may thereby increase (Conrady et al., 2011; De Tiege, Rozenberg, & Heron, 2008; Itzhaki & Wozniak, 2008; McKie, Brown, MacLean, & Graham, 1998). Although there is a great deal of knowledge about the antigen-antibody (immune) mechanism produced by B and T lymphocytes and the

inflammatory cytokines, such as interferon-α, interleukin-6 and chemokines (Figure 1), the pathogenic effect in autoimmune-inflammatory response is not well understood. Limited research has been conducted with human subjects and animal models to examine the interaction between infectious agents such as herpes viruses and the immune system and their effect on inflammation and potentially disease development, making it difficult to apply the theory to clinical medicine (Moynihan & Ader, 1996).

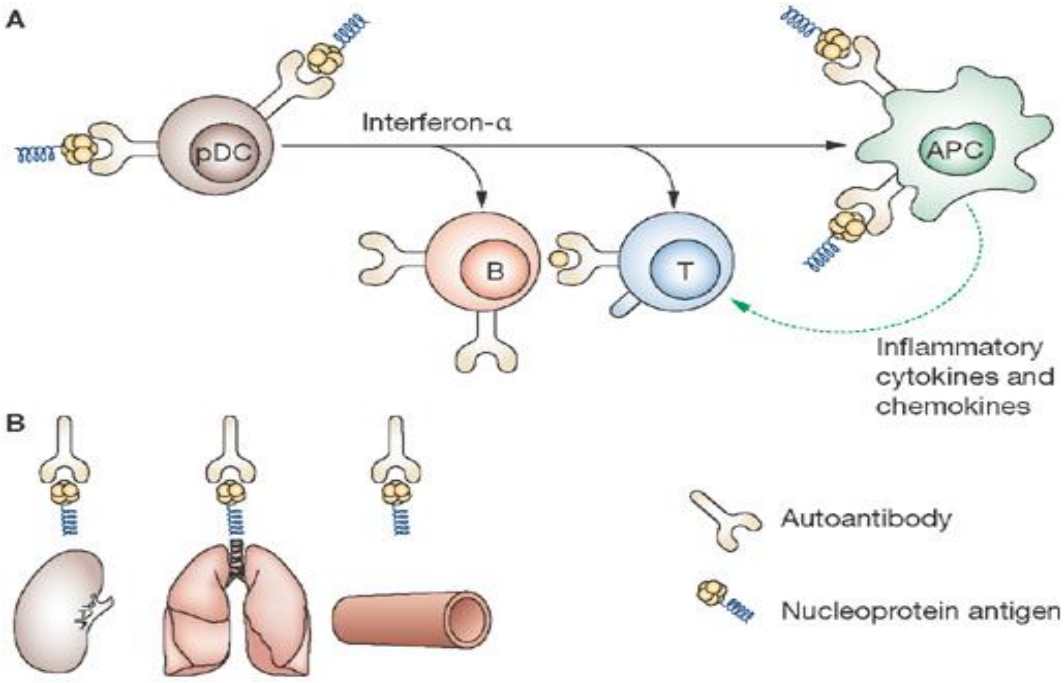

Figure 1. Nature and functions of auto-antibodies (A-inflammatory cytokines and chemokines & B-antibody & antigen response). From "Nature and Functions of Autoantibodies," by K. Elkon and P. Casali, 2008, Nature Clinical Practice Rheumatology, 4, p.12. doi: 10.1038/ncprheum0895 Adapted with permission of the author.

Innate Immunity, Inflammation, and Acute-Phase Response

Research studies on one of the most studied human herpes virus, HSV type 5 (HHV-5), also known as cytomegalovirus (CMV) indicate that this specific virus strand may be dependent on immune activation as it has been observed in tissues targeted by the immune system (Stevens, 1989; Steiner,1995). The ability of this virus to control immunological functions by activating T cells, a type of white blood cell that plays an important role in the immune system, and to produce cytokines for the virus to reactivate from its latent phase in macrophages suggests that it may play a causative role in the development of cancer, CVD, and autoimmune diseases (Soderberg-Naucler, 2006).

Soderberg-Naucler (2006) illustrated how CMV may utilize inflammation as a mechanism to reactivate itself, then spread to other cells in the inflamed tissue, and through its ability to affect numerous cellular and immunological functions, activates the innate immunity causing disease (Figure 2). A similar mechanism was observed by Pickup (2004) in examining the role of inflammation and the activation of the immune system in the pathogenesis of T2DM. This epiphenomenon has been observed in inflammatory diseases such as multiple sclerosis, diabetes, and cancer (Pickup & Crook, 1998; Soderberg-Naucler, 2006). Additionally, these diseases may provide a microenvironment that may in turn reactivate the virus triggering and sustaining inflammation (Soderberg-Naucler, 2006). While the innate immune system is the body's main defense against external threats such as infections, inflammation also functions as

the local protective response to tissue injury, which acute-phase reactant proteins such as CRP, cytokines IL-6, and TNf-alpha assist with limiting injury and healing tissue (Cone, 2001; Gabay & Kushner, 1999; Muller et al., 2002). An increase in these and other acute-phase responses induced by inflammatory cytokines when having fever or lethargy, have been observed in diabetes, atherosclerosis, arthritis and cancer patients (Pickup, 2004; Soderberg-Naucler, 2006).

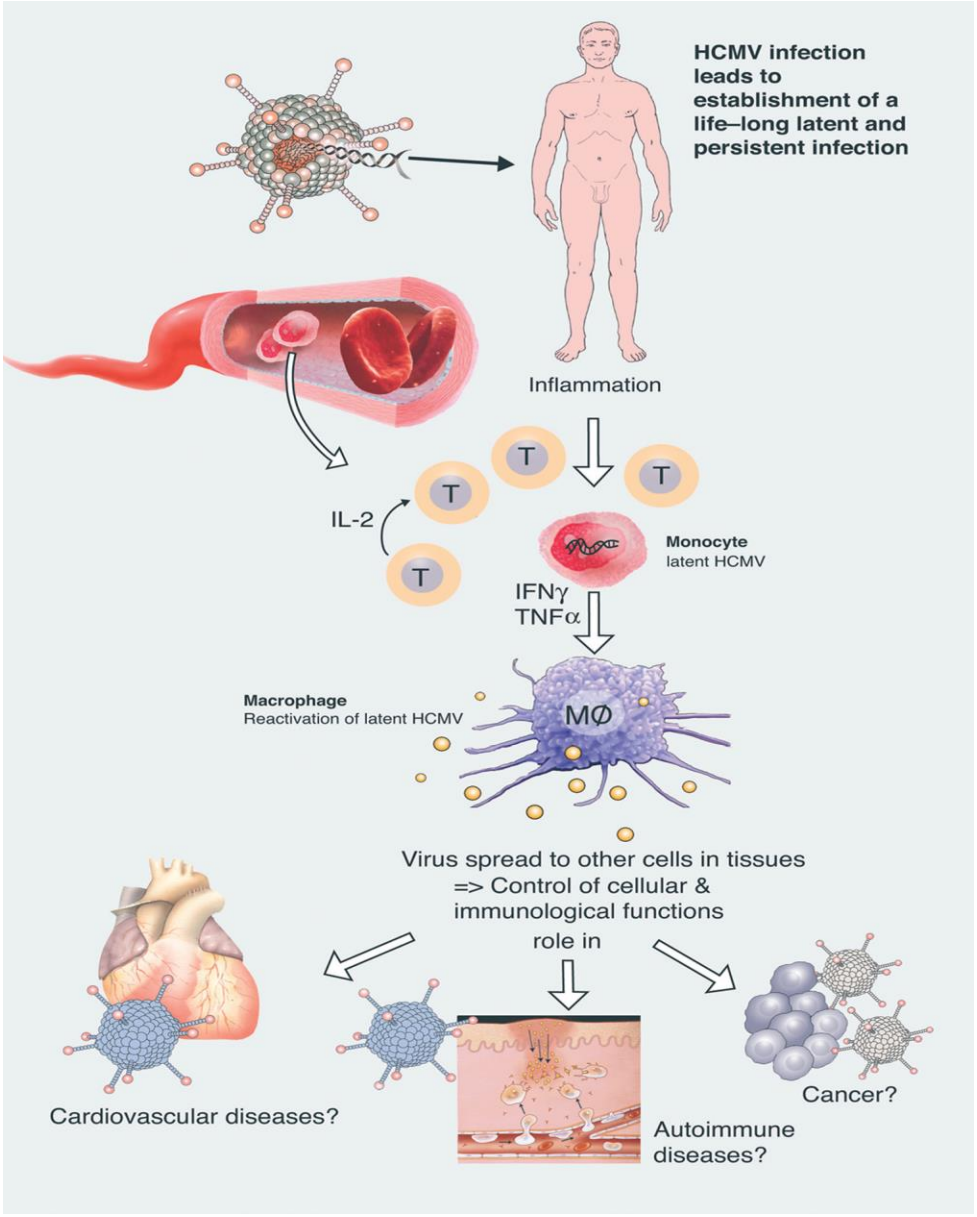

Figure 2. Mechanism between inflammation, infection and immunological functions. From "Does Cytomegalovirus Play a Causative Role in the Development of Various Inflammatory Diseases and Cancer?" by C. Soderberg-Naucler, 2006, Journal of Internal Medicine, 259(3), p. 223. doi:10.1111/j.1365-2796.2006.01618.x. Adapted with permission from John Pick Up. Adapted with permission of the author.

Relationship Between Pathogens and Inflammatory Diseases

The association between human diseases and infectious agents has been widely documented. Alzheimer's disease, for example, has been associated with various pathogens including HSV-1, Chlamydia pneumoniae, and Helicobacter pylori (Karim, 2014; Kountouras et al., 2009; Shima, Kuhlenbaumer, & Rupp, 2010). HSV-1 has been linked to Alzheimer's patients who have the APOE-4 form of APOE gene, which enables HSV-1 to enter into the brain cells (Itzhaki et al., 2008). Atherosclerosis, CHD and Asthma, particularly adult-onset asthma has been associated with Chlamydia pneumoniae and HSV-1, and the latter with rhinovirus (Gern, 2009; Hahn, Dodge, & Golubjatnikov, 1991; Prager et al., 2002; Roivainen et al., 2000). Further, approximately 20% of all cancers have been linked to pathogenic agents (Pisani, Parkin, Munoz, & Ferlay, 1997). Human papillomaviruses (HPV) have been associated with several type of cancers, including anal and colorectal cancer, breast cancer, cervical cancer, lung cancer, and some skin carcinomas (Abramowitz et al., 2010; Bosch, Lorincz, Munoz, Meijer, & Shah, 2002; Burnett-Hartman, Newcomb, & Potter, 2008; Joh et al., 2010; Lawson, Gunzburg, & Whitaker, 2006).

Similar to HSV-1, CMV or HHV-5, is often acquired during childhood, and affects the majority (over 70%) of the world's population (Britt & Mach, 1996; Onorato, Morens, Martone, & Stansfield, 1985). As with most herpes viruses, CMV can spread through bodily fluids including saliva, breast milk, semen, blood and tissue transplants (Britt et al., 1996). Once acquired, CMV can persist and live as a latent infection in the host (Onorato et al., 1985). In a study conducted by Soderberg-Naucler and colleagues

(2006), a high prevalence of CMV was found in most of the HIV-infected patients who received organ or bone marrow transplants. CMV has also been associated with long-term complications such as atherosclerosis, restenosis, transplant vascular sclerosis (TVS), rheumatoid arthritis, and systemic lupus erythematosus or SLE (Grattan et al., 1989; Torok-Storb, Fries, Stachel, & Khaira, 1993; Zhou et al., 1996). CMV has also been associated with several cancers, dementia, lupus, metabolic syndrome, myocardial infarction, and lately it has been associated with T2DM (Barzilai et al., 2007; Dziurzynski et al., 2012; Gabrylewicz et al., 2003; Nabipour, Vahdat, Jafari, Pazoki, & Sanjdideh, 2006; Rider, Ollier, Lock, Brookes, & Pamphilon, 1997; Roberts & Cech, 2005a). Moreover, CMV is one of the most common birth defect causing infections influencing mental retardation and hearing loss (Fowler et al., 1992; Onorato et al., 1985).

Chen and colleagues (2012) examined the prevalence of CMV among older adults and showed that seropositive cases of CMV were also T2DM positive (17.2% vs 7.9%, (p = 0.016) with significant high levels of HbA1c (p = 0.014). These findings give basis to suggest that CMV and T2DM may be linked by the autoimmune inflammatory response, which may increase susceptibility to the virus or alternatively increase the risk of disease development (Jun & Yoon, 2003). This also may be an indication that inflammation may act as a mediator in disease development leading to believe that there is no link to pathogenic etiology when in fact there may be (Jun & Yoon, 2003; Lecube et al., 2004 Mehta et al., 2003; Roivainen et al., 2000; Zhu et al., 2001). The link between infectious diseases and T2DM, CVD, coronary artery disease (CAD) and atherosclerosis,

however, has been controversial given that the cause of most of these conditions are considered multifactorial (Jun et al., 2003; Luscher, Creager, Beckman, & Cosentino, 2003; Zhu et al, 2001). Hypertension, obesity, smoking, hypercholesterolemia, and genetic predisposition are some of the common risk factors associated with most of these conditions (Fong, 2000; Ross, 1999). Obesity, for example, is known to play an important role in insulin resistance, diabetes and atherosclerosis (Fernandez-Real, Ferri, Vendrell, & Ricart, 2007; Karjala et al., 2011). Nevertheless, studies conducted with CAD patients showed no association with none of the traditional risk factors while their atherosclerosis appeared to have started in early childhood (Nieto, 1998; Ridker, 1999). These results support the premise that atherosclerosis may have a pathogenic etiology and chronic inflammation may be a precedent mechanism in the development of the disease. Further examination conducted by Fong (2000) showed epidemiological, pathological, and biological evidence supporting the association between CVD and infectious agents including Chlamydia pneumoniae, cytomegalovirus (CMV), HSV, and Helicobacter pylori (H. pylori). However, some of the association between CMV and CVD were found to be weak and in some studies controversial (Fong, 2000). The link between Chlamydia pneumonia (C. pneumonia) and CVD, however, seems to be well established (Ossewaarde, Feskens, De Vries, Vallinga, & Kromhout, 1998). The ability of infectious agents to induce inflammatory mechanisms and immune response has been widely demonstrated experimentally (Muhlestein, & Anderson, 2003).

Inflammation and Activation of Innate Immunity in Type 2 Diabetes

There is a great deal of evidence that suggests inflammation is involved in the pathogenesis of T2DM (Navarro & Mora, 2005; Pickup & Crook, 1998; Schmidt et al., 1999; Temelkova-Kurkschiev et al., 2002). Yet, the role of innate immunity and inflammation in the development of the disease is still uncertain (Saito et al., 2000; Schmidt et al., 1999; Snijder et al., 2003; Spranger et al., 2003). Given that the innate immune system is the body's main defense against external threats including infections, activation of innate immunity may explain some of the different clinical and biochemical conditions in T2DM patients (Fearon, 1997). These include obesity, hypertension, accelerated atherosclerosis, dyslipidemia, and limb ischemia. Inflammatory markers such CRP and interleukin-6 as well as traditional risk factors used to predict T2DM and CVD such as age, inactivity, nutrition, smoking, stress, and low-birth weight have also been identified as innate immunity activators (Freeman et al., 2002; Pradhan, Manson, Rifai, Buring, & Ridker, 2001). Pradhan and colleagues (2001) prospective and nested case-control study aimed to determine the role of inflammatory markers interleukin and CRP in the development of T2DM. One hundred eighty-eight women from a national sample of 27,628 healthy middle-aged women participated in the clinical study and were followed since 1992. Elevated levels of CRP and IL-6 were observed among those who developed diagnosed T2DM over a 4-year follow-up period. Baseline levels of IL-6 and CRP ($P < .001$) were significantly higher among women who developed T2DM than among those nondiagnosed. The relative risks for developing T2DM among women with high levels of these inflammatory markers were 7.5 for IL-6 (95% CI, 3.7-15.4) and 15.7

for CRP (95% CI, 6.5-37.9). Associations persisted after adjustment for traditional risk factors including fasting insulin levels, smoking, exercise, alcohol use, BMI, and family history of diabetes. A cross-sectional study conducted by Gregor & Hotamisligil (2011) with nondiabetic subjects and individuals with impaired glucose tolerance found a positive correlation between plasma insulin concentration, triglycerides, body mass index (BMI) and inflammation-induced acute-phase reactants. Elevated acute-phase markers have also been observed in clinical studies conducted with diabetic patients in comparison with nondiabetic subjects (Sriharan et al., 2002). Similar metabolic changes were reported by Richardson & Tayek (2002) in T2DM patients with systemic injury caused by trauma, infection, or cancer. These findings may imply that the injury response may be a factor in insulin resistance or increase in glucose production Temelkova-Kurktschiev, Henkel, Koehler, Karrei, & Hanefeld, 2002). Similarly, cytokine-induced insulin resistance, may increase the acute-phase response increasing the risk for T2DM and CVD (Fernandez-Real & Ricart, 1999). Concentrations of CRP, fibrinogen, and plasminogen activator inhibitor-1 (PAI-1) were studied with 1,047 nondiabetic subjects enrolled in a longitudinal Insulin Resistance Atherosclerosis Study (Festa, D'Agostino, Tracy, & Haffner, 2002). Results from this study indicated significant increased levels of these inflammatory markers among T2DM cases than among no converters also known as controls (fibrinogen $p = 0.013$; CRP = 0.0001; PAI-1 $p = 0.0001$). PAI-1 was found to be an independent risk factor for T2DM.

In his work, Pickup (2004), demonstrates the interaction between the different mechanisms and factors known to be activators of the innate immune system interacting

with cytokine production, which might leads to insulin resistance and other components of the metabolic syndrome (Figure 3). This mechanism is of particular interest since activated innate immunity might be a possible common antecedent of both T2DM and atherosclerosis (Pickup & Mattock, 2003). Another mechanism examined by Pickup (2004) is the fetal and neonatal programming, which suggests that prolonged environmental stressors may cause genetic mutations which may alter normal physiological responses to stress and other environmental exposures causing disease in individuals with a higher propensity to a hyper-responsive innate immune system. This theory is supported by the premise that low birth weight has been linked to high levels of acute-phase reactants in adults (Barker, 1995); therefore, it is assumed that acute-phase reactants may be precursors to certain types of autoimmune-inflammatory disease (Phillips et al., 2000).

Genetics and race have also been examined as possible mechanisms in the development of T2DM. In a study conducted by Pannacciulli and colleagues (2002), higher levels of CRP were recorded in women with family history of T2DM compared to those without a family history. When looking at the effects of race in the acute-phase response, higher concentrations of serum sialic acid have been reported in Asian diabetic patients with Indian descendant compare to White diabetic subjects (Pickup, Chana, Mattock, Samuel, & Mather, 1996). Another mechanism considered to influence the development of T2DM is individual diet, which may contribute to the activation of the innate immune system and chronic inflammation. Bakker et al. (2010) study demonstrates that excess and repeated dietary intake of certain high fatty foods may

affect the production of proinflammatory cytokines. In fact, prior studies conducted with diabetic subjects have shown a relationship between elevated levels of CRP and acute-phase reactants (TNF-alpha and IL-6) and obesity (Vlassara et al., 2002).

Further, several studies have linked a number of inflammatory makers with specific ethnic groups and gender. In a study conducted with a middle-aged population diagnosed with T2DM, Schmidt et al. (1999) found a series of markers including white blood cell count, low serum albumin, fibrinogen, alpha 1-acid glycoprotein, and sialic acid, which have also been used to predict CVD (Kannel, Wolf, Castelli, D'Agostino, 1987; Meade, 1997; Wannamethee et al., 2002; Yarnell et al., 1991). CRP has been observed predominantly in the older Scottish (Freeman et al., 2002) and Japanese population (Nakanishi, Yoshida, Matsuo, Suzuki, & Tatara, 2002) and German men (Spranger et al., 2003; Thorand et al., 2003). Both, CRP and IL-6, have been found mainly in women (Pradhan, Manson, Rifai, Buring, Ridker, 2001; Snijder et al., 2003) whereas white blood cell count have been observed in Pima Indians (Vozarova et al., 2002). CRP, fibrinogen, and plasminogen activator inhibitor-1 (PAI-1) have been associated with multiethnic subjects with insulin resistance and atherosclerosis (Barzilay et al., 2001; Duncan et al., 2000; Festa, D'Agostino, Tracy, & Haffner, 2002).

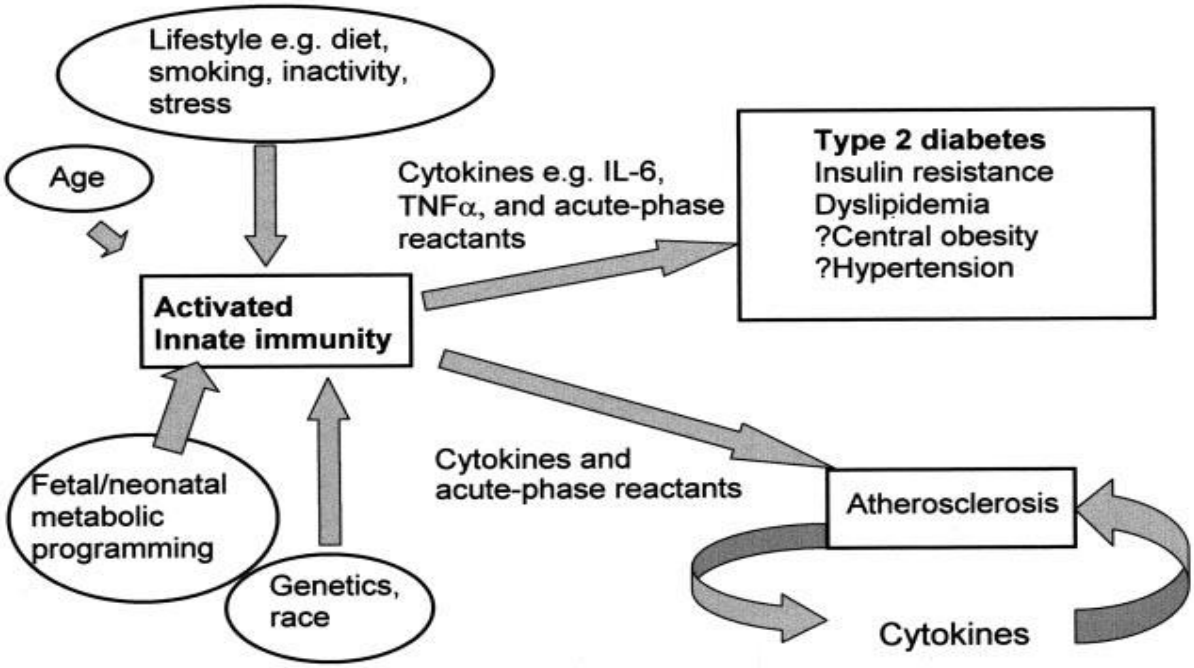

Figure 3. Activators of the innate immune system, insulin resistance, T2DM and atherosclerosis. From "Inflammation and Activated Innate Immunity in the Pathogenesis of Type 2 Diabetes" by J.C. Pickup, 2004, Diabetes Care, 27(3), p. 816. doi:10.2337/diacare.27.3.81. Adapted with permission of the author.

Inflammation and Activation of Innate Immunity in Cardiovascular Disease

Inflammation has also been associated with the pathogenesis of atherosclerosis (Blake & Ridker, 2002; Galkina & Klaus, 2009). Inflammatory markers previously associated with T2DM including CRP, fibrinogen, TNF sialic acid, and cytokines have also been found to increase the risk for myocardial infarction, stroke, peripheral vascular disease, and CVD mortality (Libby, Ridker, & Maseri, 2002; Ridker, 1999). Most risk factors such as hyperglycemia, dyslipidemia, and alterations in inflammatory mediators closely related to insulin resistance have also been observed in patients with CVD (Erdmann, 2005). Furthermore, it has been documented that serum sialic acid predicts cardiovascular mortality in T2DM patients independently of atherosclerosis

predisposition (Pickup et al., 1995). This findings suggests that innate immune response is a common precedent in atherosclerosis and diabetes. Thus, the acute-phase responses inherent to T2DM may promote the acceleration of atherosclerosis in diabetic patients (Pickup & Mattock, 2003). However, inflammatory markers have been observed in diabetic subjects without atherosclerosis and other vascular complications (Schmidt et al., 1999). Yet, other manifestations and complications of T2DM including the presence of infectious agents, might further enhance inflammatory response (Crook, Tutt, Simpson & Pickup, 1993). Additional evidence indicates that diabetic patients with high incidence of atherosclerosis suffer from impaired immune response to pathogens leading to a greater risk for infection (Heymann et al., 2008). Epstain and colleagues (1999) studied patients who were seropositive to CMV and found an odd ratio of 1.3 ($p = 0.7$) and elevated CRP levels (odds ratio 2.3, $p = 0.2$) indicating a higher prevalence of CAD in patients who had combined CMV and elevated CRP levels (odds ratio 4.3, $p = 0.01$). Findings also showed a relation between infection, inflammation and CAD influenced by gender. In men, prior CMV infection was not associated with CAD, but rather with high levels of CRP. In women, the opposite effect was observed. Prior CMV infection was found to be independently predictive of CAD (Epstein, 2002).

The relation between chronic inflammation and pathogen burden in relationship to chronic diseases has also been examined. Lutsey and colleagues (2009) used a random sample of 1,000 subjects, ages 45-84, from the Multi-Ethic Study of Atherosclerosis (MESA) to determine the cross-sectional relationship of five pathogens including C.pneumoniae, CMV, H. pylori, hepatitis A and HSV and T2DM. Results from this study

showed a higher prevalence of diabetes among subjects with a pathogen burden greater than three. No association was observed between individual infection agents and diabetes status suggesting no etiology role of infectious pathogens and the development of diabetes. However; a similar study was conducted with MESA to examine the association of cardiovascular pathogen burden and immune response in relation to socioeconomic and psychosocial gradients. Although the role of infection in CVD, particularly in atherosclerosis remains elusive, this study showed increased rates of CVD among subjects with a higher pathogen burden and low socioeconomic levels and chronic stress (Aiello et al., 2009). These findings suggest that a high burden rather than a single pathogen may be associated with increased risk of infection-related inflammation and cardiovascular damage (Zhu et al., 1999; Zhu et al., 2001). Further, stress has been linked to virus reactivation, which in turn flare up inflammation causing a dysfunctional immune response and leading to cardiovascular disease (Binkley et al., 2013).

A literature review examining the pathogenic mechanisms between psoriasis and CVD, particularly atherosclerosis, indicated that both diseases fused into inflammation (Ghazizadeh, Shimizu, Tosa, & Ghazizadeh, 2010). When inflammation is induced, T-cells are activated, chemokine and cytokine cells are produced resulting in the formation of psoriatic or atherosclerotic plaque. Similarly, a cross-sectional study conducted by the Netherlands Institute for Health Sciences Research compared patients with inflammatory arthritis (n = 1,518), patients with T2DM (n = 11,959), patients with osteoarthritis (n=4,040) and controls (n = 158,439) to determine if there was an association between an increased CVD burden on patients with selected inflammatory diseases. Study findings

showed a significantly moderate to high prevalence of CVD in arthritis cases OR = 1.5 [1.2-1.9]) and T2DM cases OR = 1.3 [1.2-1.4] (Nielen et al., 2012). These findings demonstrate that these conditions share common underlying mechanism, which also lead to the assumption that inflammation might increase the risk for developing common chronic inflammatory diseases.

Epidemiology of Herpes Viruses

Herpes simplex virus infections are prevalent worldwide (Malkin, 2004; Smith & Robinson, 2002). HSV-1 is more common than HSV-2 (Bradley, Markowitz, Gibson, McQuillan, 2014; Lafferty, 2002; Looker, Garnett, & Schmid, 2008). HSV-1 is acquired in early childhood with prevalence rates up to 40% by five years of age and by adulthood, HSV-1 affects over 60% of the population in the U.S. (Chayavichitsilp, Buckwalter, Krakowski, & Friedlander, 2009; Kimberlin,2014: Liesegang, 2001; Xu et al., 2006). Seroprevalence are similar worldwide; however, rates vary by country (kimberlin, 2014; Malkin, 2004; Smith & Robinson, 2002). However, in the United States, about one in six Americans (16.2 %) between the ages of 14 and 49 are infected with HSV-2. HSV-2 seroprevalence estimates are almost two times higher among women (20.9%) than in men (11.5%); over three times higher among blacks (39.2%) than in whites (12.3%); with African American women disproportionately affected (48%) (CDC, 2010b; Fanfair et al., 2013; Johnson et al., 1989). In 2003, over 500 million people worldwide were estimated to be infected with HSV-2, with the lowest prevalence rates in Europe and highest in Africa (Looker et al., 2008).

Pathogenesis of Herpes Viruses

Herpes viruses are among the top leading cause of viral disease in humans of public health significance (Todar, 2009). These viruses have the ability to cause acute disease or remain latent, and reactivate over and over during the lifetime of the host (Roizman, Knipe, & Whiteley, 2007). HSV-1 and HSV-2 are responsible for causing infections in the orofacial region, central nervous system, and the anogenital region (Taylor, Brockman, McNamee, & Knipe, 2002). HVS-1 is spread through saliva of an infected person, mainly through common cold sores and blisters, while HSV-2 is spread mostly through sexual contact (Sacks et al., 2004; Taylor, Brockman, McNamee, & Knipe, 2002). Both HSV species are ubiquitous and highly contagious regardless of symptomology (Kesson, 2001).

The biology of herpes viruses is described by Holmes (1999) in his book, (3rd ed) Sexually Transmitted Diseases. These viruses belong to the family of DNA enveloped viruses known as Herpesviridae. Holmes (1999) explains that humans are natural reservoirs for HSV, which could cause mild to severe conditions in humans including encephalitis and meningitis. Conversely, the viral membrane of these viruses is sensitive to an array of chemicals and substances and would not survive in harmful environmental conditions (Hunt, 2010). In fact, a cell with a damaged lipid envelop is not be infectious and transmission from cell to cell is necessary for the virus to survive (Hunt, 2010). Nevertheless, these viruses have the ability to bind to the cell surface; fuse into the cell membrane and the inner nucleus where DNA replication occurs; encode viral proteins and synthesize viral DNA genome to replicate itself (Mingo, Han, Newcomb, & Brown,

2012; Shimomura, 2008). If the virus cannot synthesize viral proteins and enzymes in host cells or the host cells do not allow such replication due to the cell's own composition, the virus would not be able to replicate (Mingo et al., 2012). Yet, herpes viruses are ubiquitous opportunistic pathogens. They have the ability to infect lymphocytes or epithelial mucosal cells; travel to and stay latent in nucleated neuron; and continue to reactivate at the same site of the initial infection (Mingo et al., 2012).

Latency, which means that the virus remains dormant in the nerve cells, also plays an important role in the survival of HSV and host immune suppression (Steiner, 1995). Most adults carry HSV in its latent's phase (Steiner, 1995). Once the virus cells are present in the body, antibodies are released, and T cells and macrophages are activated to kill infected cells (Khanna, Bonneau, Kinchington, & Hendricks, 2003). Given that HSV can infect almost any human cell, cell-mediated immunity for suppressing the infection is of great importance (Khanna et al., 2003). This immune response is normally followed by an inflammatory response that leads to some disease symptoms (Huang, Xie, Xu, Li, & Zao, 2011). Other known factors that might trigger a recurrence of the viral infection and may contribute to disease symptoms include stress, exposure to sunlight, heat, among others (Huang et al., 2011).

On rare occasions, herpes infections can result in serious sequelae and even life-threatening complications including meningitis and encephalitis and other opportunistic infections such as keratitis and gingivostomatitis, particularly in neonates (Stanberry et al., 2000; Stanberry, & Rosenthal, 2002). Recent findings also suggest that HSV may increase the risk for diseases such as HIV/AIDS, cervical cancer, cardiovascular disease,

and metabolic syndrome (de Martel & Franceschi, 2009; Nabipour et al, 2006; Roivainen et al., 2000; Zhu et al., 2009). As described by Hunt (2010), Sandri-Goldin (2006), and Whitley (1996), there are eight herpes viruses known to be pathogenic to humans (Table 1). These include Herpes simplex virus 1 (HSV-1), Herpes simplex virus 2 (HSV-2), Varicella zoster virus (VZV) or HHV-3, Epstein-Barr virus (EBV) or HHV-4, Cytomegalovirus (CMV) or HHV-5, Roseolovirus also known as Herpes Lymphoytopic virus or HHV-6, Roseolovirus or HHV-7, and Kaposi's Sarcoma-associated herpes virus or HHV-8.

Table 1

Herpes Viruses Known to be Pathogenic to Humans

Virus	Common Name	Subfamily
Human herpes virus 1	Herpes Simplex type 1	alpha
Human herpes virus 2	Herpes Simplex type 2	alpha
Human herpes virus 3	Varicella zoster	alpha
Human herpes virus 4	Epstein-Barr	gamma
Human herpes virus 5	Cytomegalovirus	beta
Human herpes virus 6/7	Exanthum subitum roseola infantum	beta
Human herpes virus 8	Kaposi's Sarcoma	gamma

Similar to HSV-1 and HSV-2, VZV or HHV-3 virus targets primary mucoepithelial cells, stay dormant for the life of the host in the neuron cells, and spread

through close and sexual contact with an infected person (Table 2). VZV is responsible for causing chickenpox and shingles and is considered airborne. HHV-4 targets B cells and epithelial cells, remains latent in B cells, and is spread through close contact, transplants and transfusions as well as vertical transmission (from mother to infant). Pathogenesis of this virus includes mononucleosis, Burkitt's lymphoma and other lymphoma-like clinical symptoms associated with persons living with AIDS. HHV-5 targets and stays dormant in monocyte, lymphocyte, and epithelial cells. It manifests as a mononucleosis-like syndrome and spreads through contact with saliva. HHV-6 and HHV-7 target and stay dormant primary in T cells. It has been suggested that these viruses may spread through close contact and air. Both viruses cause roseola infantum which is also known as the sixth disease that causes rashes in infants and young kids. HHV-8 targets primary lymphocyte cells and stays dormant in B cells. This type of virus causes Kaposi's sarcoma and Multicentric Castleman's Disease. The virus spreads through close and sexual contact with the infected person (Hunt, 2010; Sandri-Goldin, 2006; Whitley, 1996).

Table 2

Properties of Herpes Viruses

Human Herpes Type	Name	Subfamily	Target cell type	Latency	Transmission
1	Herpes simplex-1 (HSV-1)	Alphaherpes-virinae	Mucoepithelia	Neuron	Close contact
2	Herpes simplex-2 (HSV-2)	Alphaherpes-virinae	Mucoepithelia	Neuron	Close contact usually sexual
3	Varicella Zoster virus (VSV)	Alphaherpes-virinae	Mucoepithelia	Neuron	Contact or respiratory route
4	Epstein-Barr Virus (EBV)	Gammaherpes-virinae	B lymphocyte, epithelia	B lymphocytes	Saliva
5	Cytomegalovirus (CMV)	Betaherpes-virinae	Epithelia, monocytes, lymphocytes	Monocytes, lymphocytes and possibly others	Contact, blood transfusions, transplants, congenital
6	Herpes lymphotropic virus	Betaherpes-virinae	T lymphocytes and others	T lymphocytes and others	respiratory route
7	Human herpes virus-7 (HHV-7)	Betaherpes-virinae	T lymphocytes and others	T lymphocytes and others	Unknown
8	Human herpes virus-8 (HHV-8)	Gammaherpes-virinae	Endothelial cells	Unknown	Exchange of body fluids

Note. Types of Herpes Viruses. From "Microbiology and Immunology Online," by Hunt R. Retrieved 06/01/2013 from http://www.microbiologybook.org/virol/herpes.htmhtm University of South Carolina School of Medicine, Virology, Chapter 11 Herpes Viruses.

Immunopathological Aspects of HSV Infection

The human immune system responds by activating its natural defense system to remove detected foreign agents in the body. This defense mechanism against possible antigens may induce disease-like symptoms such as fever and inflamed glands while minimizing damage to the host (Banerjee & Rouse, 2007). However, immunopathology may occur when the host tissue is damaged by chronic immune reaction (Whitley, Kimberlin, & Prober, 2007). Recurrent lesions of HSV-2 in individuals with immunocompromised immune system are an example of a chronic immune response that can in fact increase the risk for other opportunistic conditions (Whitley et al., 2007). In fact, HSV and other herpes viruses such as HHV-6 have been identified as inflammatory-causing lesion agents and the latter with possibly increasing the risk for multiple sclerosis (Koelle & Corey, 2003; Swanborg, Whittum-Hudson, & Hudson, 2003). Prolonged and chronic inflammatory reactions can cause damage to certain tissue sites or organs. For example, HSV infections may cause impaired vision, retinitis, and ganglionitis and have been associated with diseases like arthritis and Alzheimer's disease (Pepose, 1991a, 1991b; Stuart, Summers, Morris, Morrison, & Leib, 2004). The immune response to HSV-1 involves several mechanisms including the secretion of cytokines such as NK cells, CD4+ and CD8+ lymphocytes, TNF- a, IFN-a, and IFN-y, which uphold antiviral action (Kumel et al., 1982; Linnavuori & Hovi, 1987; Weidinger et al., 2000). The ability of HSV-1 to interfere with the expression of major histocompatibility (MHC) class II

antigen during its active state and the MHC class I gene during its latent state is what allows the virus to evade the immune response; a phenomenon observed in HIV (Halford, Balliet, & Gebhardt, 2004; Sawtell, Thompson, & Haas, 2006).

Association Studies Between Herpes Simplex Viruses and Diabetes

Although limited, the association between T2DM and CVD-prone pathogens have been examined in prior studies (Roivainen et al., 2000; Zhu et al., 2001). Yuhua (2005) and associates studied the relationship between HSV-1 and T2DM among 1,566 diabetic and nondiabetic subjects. Results indicated a significant association between HSV-1 and T2DM. Given that chronic inflammation is involved in the pathogenesis of T2DM, it is assumed that inflammation is flare up by an infectious agent such as HSV-1 (Pickup, 2004). A recent study was conducted by Oke & Oke (2014) to investigate the prevalence of HSV-1 and HSV-2 in diabetic and nondiabetic patients in a diabetic clinic in Nigeria. The study population consisted of 320 patients aged 35-50. The prevalence of hyperglycemia in this study was approximately 85% with fasting blood sugar (FBS) ranging from 125 to 560 mg/dl. Approximately 50 (16%) had a normal glycemic reading ranging from 55-90 mg/dl. Among the 270 diabetic patients, 55 (20.4%) were diagnosed with ketonuria (as a result of insufficient insulin) and were seropositive to both, HSV-1 and 2. Diabetic patients with no ketonuria were also seronegative for HSV-1 and HSV-2. This study shows a significant association between HSV-1 and 2 seropositivity and diabetes; particularly among diabetic patients with hyperglycemia and ketonuria. Ketosis-prone T2DM is one of the most common forms of diabetes in African populations since 1987, which researchers believe its onset is trigger by viral infection (Sobngwi et al.,

2008; Tesfaye, Cullen, Wilson, & Woolley, 1991; Jun et al., 2003). These claims were also supported by Tesfaye and associates (1991) who reported cases of diabetic ketoacidosis induced by genital herpes. Similar results were reported by Sobngwi and colleagues (2008) who conducted a study to determine if an association between ketosis-prone T2DM and other herpes viruses exists. A study population consisting of 187 (81 ketosis-prone T2DM and 106 nonketosis T2DM) black patients of African origin were compared to 90 nondiabetic control group. A high prevalence of ketosis-prone T2DM and a very high prevalence (almost 6-fold higher) of HHV-8 infection among patients with ketosis-prone T2DM (approx. 87.7%) was observed compared to a lower prevalence of HHV-8 among nonketosis T2DM (15.1%) OR 39.9 [95% CI: 17.1-93.4] ($P < .001$) and among the control group (40%) OR 10.7[95% CI: 4.9-23.4] ($P < .001$).

The association between herpes virus entry mediator (HVEM), a receptor for HSV and cytokines, was studied in 840 obese individuals by Bassols et al. (2009). HVEM gene expression was found significantly higher in obese than in nonobese individuals ($p < 0.0001$). These results indicate a positive relationship between HVEM and obesity suggesting that this receptor may be a causal factor in the pathogenesis of obesity and inflammatory response (Bassols, Moreno, Ortega, Ricart, & Fernandez-Real, 2009). Additional evidence supporting these findings was documented in a retrospective cohort study conducted by Shah and Hux (2003) to determine the risk of diabetic patients to acquire an infectious disease. Researchers looked at diabetic and nondiabetic patients in Ontario Canada at two points in time 1996 and 1999. The risk for acquiring an infection among diabetic versus nondiabetic patients was OR 1.21 [99% CI 1.20-1.11]

while attributable death among diabetic patients was up to OR 1.92 [CI 1.79 - 2.05]. These results provide additional evidence on possible increased susceptibility among immune compromised individuals to infection.

Findings from a case-control study conducted by Roberts & Cech (2005b) indicated that a person previously exposed to one of the most common herpes viruses, CMV, has up to a 12 times greater chance of developing T2DM. Results also showed that diabetic patients had a greater seroprevalence of IgG CMV antibodies (97.6%) compared to nondiabetic patients (86.7%) with a statistical significance OR = 6.2 [95% CI: 1.1 – 36.0] ($p < 0.05$). Greater odds were observed in a subset of patients with diabetes who had significantly more vascular complications than the controls OR = 12.4 [95% CI: 1.3 to 117] ($p < 0.05$). A linear trend between diabetes, seropositivity to CMV, and age was also observed in this study. Given the association of atherosclerosis with diabetes and CMV, these findings support the premise that CMV may be a common factor between diabetes and CVD.

Association Studies between Herpes Simplex Viruses and Cardiovascular Disease

To examine the relationship between herpes simplex viruses and CVD, Mendy and colleagues (2013) conducted a cross-sectional study with 14,415 participants of NHANES 1999-2010, aged 20-49 years with a mean age of 34.3 years. Among the participants, over half (51.3%) were seropositive for HSV-1, 7.5% were seropositive for HSV-2 and 15.2% were seropositive to both HSVs. Results of this study indicated a significant association of HSV-2 with CVD OR 1.56 [95% CI = 1.09-2.21]; however, HSV-1 was not associated with CVD in this study OR 1.13 [95% CI = 0.79-1.62]. Yet,

HSV-1 and HSV-2 have been found in human atherosclerotic plaque (Gyorkey, Melnick Guinn, Gyorkey, & DeBakey, 1984; Friedman, Macarak, MacGregor, Wolfe, & Kefalides, 1981; Nicholson, & Hajjar, 1998). Likewise, CMV, a well-studied marker for atherosclerosis has been linked to endothelial dysfunction (Danesh, Collins, & Peto, 1997).

A prospective study conducted with 643 men to examine the association between HSV and CMV and MI and stroke indicated no evidence of these two pathogens increasing the risk for atherothrombotic. However, it has been suggested that perhaps viral particles may play a role in the initial process, but not at the clinical phase of the disease (Ridker, 1998, 1999). A population-based study with a similar sample size (515) examined the role of HSV-1 and CMV in CHD patients (Sorlie et al., 2000). A significant association between CMV and CHD with a higher risk in diabetic patients was reported in this study. However, no association was found between HSV and CHD suggesting some degree of disease-pathogen specificity (Sorlie et al., 2000). Nevertheless, while most studies conducted with CVD patients have focused on a single pathogen, it has been suggested that aggregate pathogen load may be associated with increased risk for CAD (Fernandez-Real et al., 2007). Pathogen burden has also been associated with plaque rupture and acute thrombotic occlusion, major risk factors for acute MI, as well as insulin resistance, a well-known factor for CAD (Epstein, 2002; Fernandez-Real et al., 2007). This premise has been extended by several prospective studies in which pathogen burden was found to be predictive of CVD (Zhu et al., 2001). Zhu and associates conducted a study with 124 middle-aged men to examine the effect of four pathogens: HSV,

enteroviruses, CMV, and chlamydia pneumonia, as well as insulin resistance. A strong association was observed in insulin resistance patients and seropositivity with multiple pathogens though a weak association was observed with a single pathogen including HSV. It should be noted that the small number of individuals who were seropositive for HSV-1 and HSV-2 may have limited the study's ability to find a significant association with these two infectious pathogens.

In a cohort study of 890 patients who had CAD based on angiography, however, IgG antibodies for CMV, HSV-1, HSV-2, Hepatitis A virus (HAV) and CRP levels were found significantly higher in patients who subsequently developed MI (Zhu et al., 2009).

A similar study with contradictory results was conducted with 122 post-menopausal women who tested positive for prior exposure to multiple infectious agents including C. pneumoniae, H. pylori, HSV, and CMV (Nabipour et al., 2006). Although results showed little evidence of the association between baseline serology of studied pathogens and risk for CVD, C. Pneumoniae has been implicated in chronic inflammatory response leading to atherosclerotic lesion progression in other studies (Altman, 2003; Campbell & Kuo, 2004; Grayston, Kuo, Campbell & Benditt, 1993; Wu & Wu, 2006). Equally, the longitudinal Helsinki Heart Study, a coronary prevention trial that monitor patients who suffered from myocardial infarction (MI) or coronary death during the eight and a half year trial period, examined CRP levels and antibodies for several viruses including HSV. Results from this study found higher levels of HSV-1 and Chlamydia pneumoniae antibodies in studied cases. The CRP mean was higher in cases in comparison to controls (4.4 versus2.0 mg/L: $p < 0.001$). The odds ratio for cases with

high antibodies and CRP levels were 2.4 [95% CI 2.9-3.3] for HSV-1 and 5.4 [95% CI2.4-12.4] for Chlamydia pneumoniae. The relative risk for high levels of antibodies for HSV-1 was 2.05 (95% CI 1.15-3.67) in nonsmokers and 23.74 for smokers [CI1.58-8.86]. These findings demonstrate that high levels of antibodies of HSV-1 may be a marker for active, recurrent infection and a potential trigger for inflammation reaction increasing the risk for coronary heart disease (Benditt, Barrett, & McDougall, 1983; Gyorkey et al., 1984; Hoffmeister et al., 2001; Ridker et al., 1998; Roivainen et al., 2000).

A few seroepidemiological studies have also linked HSV-2 as a potential cardiovascular pathogen; particularly, it has been implicated in atherosclerosis, CAD, and MI (Espinola-Klein et al., 2002; Nicholson et al, 1998; Rupprecht et al., 2001). HSV-2, however, have been associated with carotid wall thickening, vascular disease, thrombogenic and atherogenic of host cells (Sorlie et al., 1994). These findings have been extended by recent prospective studies specific to HSV-2. Sun et al (2004) investigated the relationship between HSV-2 and hypertension by inducing inflammation, a co-factor of HSV, in a cross-sectional study of 1,244 patients. The prevalence of HSV-2 IgG seropositivity was significantly higher in subjects with hypertension than in the control group (38.3% vs 29.8%, $p = 0.002$) and found to be an independent risk factor for hypertension (Sun et al., 2004). Similar results were found in a study of 31 patients undergoing coronary artery bypass. An association between inflammatory cells, cold sores, and HSV-2 antigen was observed in biopsies (Sun et al., 2004).

Summary

This literature review explored research in some areas of chronic diseases, autoimmune inflammatory response and infectious agents including those tested in the present study, HSV-1, HSV-2, and CMV. Current evidence demonstrates that inflammation is a co-factor of both, chronic disease and infection. Although the role of pathogens in the development of chronic disease is not well understood, studies that examined the underlying autoimmune responses of infection suggest an increased risk for developing inflammatory diseases. Alternatively, persons with chronic diseases may develop susceptibility to infection. Despite the accumulating evidence that infectious agents may predispose individuals to the development of chronic diseases, seroepidemiological studies continue to fail in demonstrating a strong causality or association between a single or multiple pathogens and disease development. Recent studies have hypothesized that if infection causes T2DM or CVD, it is likely to be caused by multiple pathogens. Other controversial area that remain to be explored is whether multiple agents or recurrence of inflammation influence early infection.

In summary, conflicting results found in the current literature pose a challenge when it comes to synthesizing results and generate inferences that apply to the general population. There is urgency for additional clinical research and longitudinal studies to better understand the activation of the immune system in response to inflammatory-induced agents and/or mechanisms. Although some preliminary clinical research is beginning to explore some possible mechanisms by which inflammation can be linked to pathogenic response or a chronic condition, causal factors need to be established. The

design for the present study was chosen based upon the existing literature relevant to this study and data available and would not allow for determining causality. The next chapter provides a description on methodology, instrumentation, study population, sample size, and analysis used in this study.

Chapter 3: Research Method

Study Design and Approach

In this study, a population-based, cross-sectional design was used to determine whether there was an association between two major chronic diseases, T2DM and CVD and two herpes viruses, HSV-1, HSV-2. To evaluate whether burden of herpes infections (having two or three herpes viruses) has an effect on T2DM or CVD, CMV was added to the analysis of burden of infections. In addition, the relationship between (a) T2DM and CVD and (b) selected inflammatory markers (CRP, homocysteine, and ferritin) was examined. The primary risk factors for both diseases, as identified in the literature review, were also evaluated in relationship to the dependent variables under study. Twelve years of secondary data (1999-2010) were analyzed to evaluate disease prevalence, trends, and associations. Although a cross-sectional design cannot determine a causal relationship, this study was expected to add to the literature on the potential association between the tested variables. Given the nature of the data, a nonexperimental, correlational approach was used. Study participants were randomly selected by a national survey and information on variables of interest were collected through three different means: a self-reported questionnaire, a physical examination, and clinical data for each of

the participants. These three data collection methods provided a means to confirm the status of the disease and to validate the data.

Data Collection and Sample

This study used the NHANES data collected from 1999–2010. NHANES, which began in 1999 and is conducted every 2 years, is a national probability survey conducted by the National Center for Health Statistics, Centers for Disease Control and Prevention (CDC, 2012). The survey is designed to estimate the prevalence of common chronic conditions and associated risk factors in service of chronic disease prevention. NHANES data are the product of a combination of (a) demographic, nutrition, and health-related questions and (b) physical examinations that consist of laboratory tests and medical, dental, and physiological measurements that are made by highly trained medical personnel.

A national representative sample consisting of approximately five thousands U.S. residents is collected by NHANES each year using a complex, multistage, cluster design. Health topics collected by NHANES include but not limited to cardiovascular disease, kidney disease, obesity, oral health, physical fitness, and sexually transmitted diseases (CDC, 2012). A total of 99,806 participants were included in NHANES from 1999-2010, from approximately 89 strata (selected surveyed areas) with 2–3 household units—or primary sampling units (PSUs)—in each stratum. The final study sample consisted of 6,706 individuals, aged 20-49, for whom interviews and medical examinations were completed, and for which laboratory results were available for all variables of interest.

Selection of NHANES Participants and Setting

NHANES participants are selected through an algorithm that randomly selects neighborhoods based on census information. Approximately 15,000 households are screened, 3,500 are selected, and 5,000 residents from the selected households are interviewed. Residents of household units are randomly selected and approached by interviewers (health, research and academic professionals) to determine their eligibility. If a resident agrees to be a part of the study they are scheduled for an interview. The interview is conducted in their home and last approximately one hour. Responses are recorded on a lap top computer. Individuals 60 and older, African Americans, Hispanics and Asians are oversampled in order to increase representation of their population and sample reliability (CDC, 2012). Participants also undergo a physical examination by a team of health technicians, dentists and doctors in the Mobile Examination Center (MEC) which consists of four connecting trailers containing high-tech medical equipment. Health exams are given which include measurements of bone density, blood pressure, weight and height. Urine and blood samples are collected and tests for health conditions such as STIs, anemia, substance use and exposure to environmental chemicals.

Selection Criteria for Study Participants

NHANES serological test results for HSV-1 and HSV-2 were available for individuals 14-49 years old. Serological data for adolescents 14-17 are restricted and not available for public use. Additionally, some of the variables in question were collected solely from individuals 20 years and older. As a result, this study was limited to NHANES participants between the ages of 20 and 49 for which interviews, medical

examination, and laboratory results for all variables of interest were completed. NHANES participants whose answers were recorded as don't know, refused, or missing were excluded from the analysis. In addition, some analyses were limited to a specific time intervals and subgroups for which the data were available. These include antibody for CMV (data available from 1999-2004) and homocysteine (data available from 1999 to 2006).

Instrument and Materials

Secondary quantitative data from NHANES 1999-2010 were used in this study. NHANES datasets can be accessed through the CDC website in the NHANES page. NHANES begun in the 1960's, and in 1999 became a continuous program examining different health topics and behaviors among various populations. The survey is comprised of sociodemographic, dietary and health related questions and also includes physical examinations, urine and blood tests to determine the prevalence of major diseases and risk factors. The household interview component contains a screener, family interview and sample person questionnaire given to individuals in their home (CDC, 2012). The Mobile Examination Center (MEC) Questionnaires consist of a MEC Computer-Assisted Personal Interview (MEC CAPI) and MEC Audio-Computer-Assisted Self Interview (MEC A-CASI) where participants can answer questions without the interviewer being present. Data is collected using a touch screen lap top computers with electronic pens. Interviewers can easily submit information to data servers which are available to NCHS staff within 24 hours. The CAPI and CASI programs contain built-in quality assurance checks when unrealistic responses are entered. Validity and reliability

for all questionnaires are pretested before implementation (CDC, 2012). Additionally, laboratory techniques used from 1999 to 2010 to detect enzymes and antibodies to studied agents were identical and therefore comparable for trend analysis.

Justification for Using NHANES Survey Data

NHANES is a national survey which data can be used to provide a measurement of diagnosed and undiagnosed medical conditions and rates of diseases which can be useful in developing public health interventions, programs and policies. NHANES is distinctive because it combines survey data with physical examination and clinical data to assess the health of the participants and increase the validity of findings. Additionally, NHANES data has been systematically implemented since 1999 to report a U.S. representative sample of health data. The survey contains information on several different health variables which makes it appropriate for this study. NHANES findings have been used to determine disease prevalence, develop national standards and health policies and design health programs and services demonstrating its relevance and use for the population. Table 3 lists dependent, independent, and covariates included in this study.

Table 3

List of Dependent, Independent & Covariates studied, NHANES 1999-2010

Dependent Variables	Independent Variables of interest	Covariates
Diagnosed T2DM	Herpes viruses	Age
Diagnosed CVD	HSV-1 seropositive	Gender
	HSV-2 seropositive	Education Level
CVD related conditions	CMV seropositive	Race/Ethnicity
CHD		Household Income
CHF		BMI

Angina	Inflammation Markers	Smoking Status
Heart Attack	CRP	Hypertension
MI	Homocysteine	Cholesterol
Stroke	Ferritin	

Selection of Studied Variables

Selection of study variables was based on research interest available in NHANES 1999-2012 surveys. Covariates were selected based on empirical evidence on risk factors for both T2DM and CVD. Variables such as age, gender, education and race/ethnicity were collected from survey interview data. Physical examinations such as height, weight and BMI and laboratory blood tests for HSV-1 and HSV-2 antibodies were conducted by MEC (CDC, 2012). Blood was drawn from the participant's arm via venipuncture to obtain laboratory results. In the laboratory, the blood is processed, stored and shipped to different laboratories for analysis. The complete blood count (CBC) results are reported in the MEC, and all other results are reported from NCHS to the participant. The volume of blood drawn is determined by age. For 12 plus years, 115ml of blood is collected from participants. Urine samples are collected from participants 6 years old and above.

Dependent Variables

T2DM

Self-report of T2DM was assessed by the following questions: Have you ever been told by a doctor or health professional that you have diabetes or sugar diabetes? How old were you when you were told that you had diabetes or sugar diabetes?

Respondents input age at the time they were told. Additionally, participants were asked about diabetes-related medication, respondents answered yes or no to questions such as these: Are you now taking insulin?, For how long have you been taking insulin?, and Are you now taking diabetic pills to lower your blood sugar? Only respondents who answered questions with a yes or no were included in the analyses; missing values were excluded.

Clinical diagnosis of T2DM was assessed by evaluating major T2DM indicators. Blood sample measures of *plasma glucose* (500uL/1mL) and *glycohemoglobin A1C test* (400uL) were obtained from participants 12 years and older who were examined in the morning sessions. A *fasting glucose* blood test was performed in all participants 12 years and older who were examined in the morning session after a 9-hour fast. After the venipuncture, participants are asked to drink 75 milligrams of Trutol® and to have a second venipuncture two hours (±15 minutes) after the first venipuncture.

Diabetes was defined by at least one of the following criteria: (a) self-report of diagnosis by a doctor or other health professional, (b) FBG ≥126 mg/dl, or (c) HbA1C ≥6.5%. Individuals without diabetes did not meet any of these criteria

CVD

For the purpose of this study, CVD was defined as self-report of diagnosis by a doctor or other health professional of coronary heart disease, angina, heart attack, congestive heart failure, or stroke. Self-report of CVD was determined by selected questions under NHANES medical conditions. CVD questions focused on congestive heart failure (CHF), coronary heart disease (CHD), angina (also known as angina pectoris), heart attack (also called myocardial infarction or MI), and stroke.

CVD questions are as follows:

Congestive heart failure questions: Has a doctor or other health professional told you that you had congestive heart failure? How old were you when you were told that you had congestive heart failure?

Congestive heart failure questions: Has a doctor or other health professional told you that you had congestive heart failure? How old were you when you were told that you had congestive heart failure?

Coronary heart disease questions: Has a doctor or other health professional told you that you had coronary heart disease? How old were you when you were told that you had coronary heart disease?

Angina questions: Has a doctor or other health professional told you that you had angina? How old were you when you had angina?

Heart attack or MI questions: Has a doctor or other health professional told you that you had a heart attack or MI? How old were you when you had a heart attack or MI?

Stroke questions: Has a doctor or other health professional told you that you had a stroke? How old were you when you had a stroke? Only respondents who answered questions with a yes or no were included in the analyses; missing values were excluded.

Independent Variables

The primary independent variables for this study include HSV-1 and HSV-2. CMV was added as another independent variable to test the effect of burden of herpes infections (HSV-1, HSV-2, and CMV) in T2DM and CVD. Secondary independent

variables include three inflammatory markers, C-reactive protein (CRP), Homocysteine and serum Ferritin. Evidence-based major risk factors were included as covariates.

HSV-1 and HSV-2 Seropositivity

To determine the serostatus for HSV-1 and HSV-2, blood samples of 500uL were collected from individuals 14-49 years at the Medical Examination Center (MEC) and tested for both HSV-1 and HSV-2 antibodies. However, only test results from participants 18 to 49 years of age were available for public use. An enzyme immunoassay (EIA) with specific glycoprotein (antigens) to differentiate between HSV-1 and HSV-2 antibodies was used; however, data for antibodies was limited to only few years. Serostatus was recorded as positive or negative. Missing values were excluded.

HSV-1 and HSV-2 Self-reported

Self-reported for HSV-1 was not collected in NHANES. A single question to determine HSV-2 history was asked to study participants, Has a doctor or other health care professional ever told you that you had genital herpes? Due to the fact that genital herpes can be caused by HSV-2 and HSV-1, individuals who reported being told they have genital herpes were also matched with their serostatus for HSV-1 and HSV-2. NHANES clinical data does not differentiate between HSV-1 and HSV-2 for genital herpes. Missing values were excluded.

CMV

To determine the serostatus for CMV, blood samples of 350uL were collected from persons aged 6 to 49 years and tested for CMV antibodies. CMV specific antibody was measured with an ELISA assay. Analyses on CMV were limited to a time period of

six years given that data were collected solely from 1999 to 2004. Test results for participants aged 20 to 49 were available for public use and included in the study analysis. Serostatus for CMV was recorded as positive or negative. Missing values were excluded.

Inflammatory Markers

Inflammatory markers also known as biomarkers are useful clinical diagnostic and prognostic tools for ruling out specific conditions. For example, elevated levels of CRP, a protein synthesized by the liver, is an indication of inflammation present in the body and has been used as a biomarker for the risk of CVD and T2DM (Ballantyne et al., 2005; Davidson, 2011; Ridker et al., 2000; Watson et al., 2012). *Interleukin or IL-6,* a protein-coding gene, functions with inflammation in response to harmful stimuli such as infection (Ridker et al., 2000I). L-6 marker was not collected by NHANES and therefore not included in the study. *Fibrinogen,* a protein synthesized by the liver, helps to stop clots to form by converting thrombin into fibrin and may indicate a risk for diabetes. Fibrinogen data was collected for only few years and therefore not included in study analysis. Elevated levels of *Ferritin,* a protein that controls the levels of iron in the blood, may be an indication of inflammation, heart failure, diabetes and other conditions (Oshaug et al., 1995; Ridker et al., 2000). Elevated levels of *Homocysteine*, an amino acid found in the blood plasma, may increase the risk for heart disease (Ridker et al., 2000).

CRP

Approximately 300uL of blood samples from individuals 3 years and older were used to measure the levels of CRP. The following predictive values were used for CVD: Normal levels of CRP range from 0 to 1.0 mg/dl. Elevated high sensitivity CRP (hsCRP) levels associated with CVD and other chronic diseases. Any hsCRP level less than 1.0 mg/L is considered low risk; average risk is considered between 1.0 and 3.0 mg/L; and elevated hsCRP levels and highest risk are considered more than 3.0 mg/L. The median concentration of the study population was used as the cut off for both CVD and T2DM when applicable.

Homocysteine

Plasma homocysteine is a nonprotein amino acid. Blood samples of 1.25mL were tested from individuals 1 year and older. Analysis for homocysteine were limited to a time interval of eight years given that data was collected only from 1999 to 2006. For the purpose of this study, the median value of homocysteine in the study population was used as a cut off when applicable.

Ferritin

The level of serum ferritin is directly related to the amount of iron stored in the body. Blood samples of 1.25mL were tested from individuals 1 year and older. Normal values of Ferritin may vary from lab to lab and in the clinical field in general. For this study, the median value of ferritin in the study population was used as a cut off when applicable.

Covariates

Age

Age was determined in the questionnaire by the date of birth given by participants. Respondents who answered don't know or refused to answer were coded as missing and excluded from the analysis. Analyses were limited to participants ages 20-49 due to data availability.

Gender

Participants were asked in the questionnaire to select if they were male or female. Respondents who answered don't know or refused to answer were coded as missing and excluded from the analysis.

Education Level

Respondents were asked in the questionnaire: What is the highest grade or level of school that you have completed or the highest degree you have received? Education levels were divided into two categories: individuals who completed high school and those who had college level. Respondents who answered don't know, refused or the answer was coded as missing were excluded from the analysis.

Race/Ethnicity

Respondents were asked in the questionnaire: What race do you consider yourself to be? NHANES used five categories: nonHispanic White, nonHispanic Black, Mexican American, Other and Other Hispanics and could only select one. Respondents who answered don't know, refused or answer was coded as missing were excluded from the

analysis. Due to frequency distribution limitations with multi-categorical variables, race was recategorized as nonwhites versus whites for binary logistic regression.

Household Income

In the questionnaire, family was described as: Individuals and groups of individuals who are related by birth, marriage or adoption. Stepchildren, parents or siblings are included. It also includes unmarried partners if they have a biological or adoptive child in common. Participants were asked about the total income for you and your family members in the last calendar year- including income from all sources we have just talked about such as wages, salaries, social security or retirement benefits, help from relatives and so forth. Can you tell me that amount before taxes? Respondents entered the amount of income. In this study household income was categorized as follow: income from 0 to 24, 999; income from 25, 000 to 54, 999; income over 55, 000. Respondents who answered, don't know, refused or answer was coded as missing were excluded from the analysis.

BMI

The body mass index (BMI) was calculated using participants' body weight and height (weight divided by height). In this study, BMI <25 was categorized as normal weight; BMI ≤25 and <30 as overweight; and BMI≥30 was considered obese. Respondents who answered, don't know, refused, or answer was coded as missing were excluded from the analysis.

Smoking Status

Smoking status was determined by asking, have you smoked at least 100 cigarettes in your entire life? Respondents answered yes or no. They were also asked, Do you now smoke cigarettes: every day, some days or not at all? Respondents answer yes or no. Only respondents who answered questions with a yes or no were included in the analyses. Smoking status was categorized in the analysis as follows: never smoke; past smoker; and current smoker.

Hypertension /Blood Pressure

Self-reported high blood pressure of the participants was assessed by the following questions: Have you ever been told by a doctor or other health professional that you had hypertension, also called high blood pressure? Respondents answered, yes or no to questions such as Have you been told on 2 or more different visits that you had hypertension, also called high blood pressure?, because of your blood pressure have you been told to take prescribed medicine? Are you now taking prescribed medication? Respondents were also asked, what age were you told that you had high blood pressure? Only respondents who answered questions with a yes or no were included in the analyses. Physical measurements of systolic and diastolic BP consisting of up to 4 different BP readings in different time intervals were collected from survey participants. For the purpose of this study, the 2nd reading was selected as a better estimate for measuring BP among participants.

Cholesterol

Self-reported cholesterol levels were assessed. Respondents answered yes or no to the following questions: Have you ever been told by a doctor or other health professional that your cholesterol was too high? To lower your blood cholesterol, have you ever been told by a doctor or other health professional to take prescribed medicine? and Are you now following this advice to take prescribed medicine? Only respondents who answered questions with a yes or no were included in the analyses.

For physical examinations total cholesterol, HDL- cholesterol, LDL-cholesterol were measured through blood lipid levels of 1mL from individuals 12 and older. Total cholesterol was calculated from totals of HDL and LDL levels. For analysis purposes, the median of total cholesterol, HDL and LDL values in the study population were used.

Statistical Analysis

This study employs secondary data from the CDC NHANES database and utilizes a cross-sectional design, which provides the means for conducting descriptive and inferential analysis. First, simple descriptive statistics to examine the distribution of the study sample (N=6,706) such as means, standard deviations, and proportions (when appropriate) for the main outcome variables (T2DM and CVD) and explanatory variables (demographic and clinical variables) were applied. Second, a binary analysis and chi-square test was used to determine the relation between the two dependent variables and categorical predictors. Given that NHANES provides a large representative sample for conducting secondary research studies, statistical hypothesis testing power is not a concern for the analyses needed in this study. Multiple logistic and stepwise regression

analyses adjusting for demographic and clinical variables was applied for confounding factors using continuous waves of NHANES data from 1999 to 2010.

All statistical analyses were performed in SAS-Callable SUDAAN, Release 11.0.1. (RTI, 2013). A reference table that included all study variables names, SAS data files, and relevant codebook and documentation was used for accuracy and validity. NHANES data files were imported from the CDC NHANES website located at http://www. NCHS.gov and saved in a preassigned library in SAS v9.3. (SAS, 2000-2004). Once all of the needed variables were downloaded into the SAS library, data were merged by SEQN ID, recoded and categorized when necessary for analysis purposes. Analytical recommendations from NHANES for sample weights and variables of interest were used. Descriptive analysis was performed on independent, dependent, and covariate variables. When necessary and applicable, study variables were dichotomized at the median value defined by the studied population into either low to normal or average to high levels. The high and average levels of CRP were combined given that the frequency in the high group was small, and there were too many missing once the weight variable was applied. A similar approach was used with ferritin levels due to small sample size in the high and low levels of ferritin for men and women. Therefore, CRP was recategorized into high or average levels versus low or normal levels, and ferritin levels for men and women were combined and recategorized as high versus low and normal versus low in the multivariate regression analyses.

To determine the relationship between two categorical variables, a standard Pearson’s Chi-square test was performed and to determine the relationship between two

continuous variables, the standard t-test was used. To test the hypothesis of association between HSV and T2DM and CVD, logistic and stepwise regression analyses were performed (Draper, & Smith, 1981; Hocking, 1976). Adjustments were made to control for potential confounding factors and calculate the adjusted odds ratio and respective 95% confidence interval (CI). Primary independent variables and covariates were entered into four different models. In the 1st model, crude rates for all independent variables and covariates were calculated. In the 2nd model, adjusted rates for all the independent variables and covariates were calculated. In the 3rd and 4th models, the most predictive independent variables and covariates were included in each group. The 3rd and 4th models consisted of different stages. At first stage, the primary independent variable, HSV-2, was entered into the model. Then a forward stepwise regression was performed to add the most predictive demographic variables (second stage). Significant demographic variables such as age, gender and household income were added into the model at this stage. After adjusting for these variables, additional clinical variables were added into a final model (third stage). Since the final model (column 4 in odds ratio tables for T2DM and CVD) removes the effect of all possible confounders, this model provides the best effect estimates. Overall associations were represented by values $p \leq 0.10$, $p \leq 0.05$, $p < 0.01$.

The PROC RLOGIST (RTI, 2013) was used to model the risk of T2DM and CVD as a function of HSV-1 and HSV-2; HSV (the combination of HSV-1 and HSV-2 was defined as 0 = neither HSV-1 nor HSV-2, 1 = HSV-1 or HSV-2, 2 = HSV-1+HSV-2, 3 = HSV-1+HSV-2+CMV); and burden of herpes infection was defined as 0, 1, 2, 3 adjusted by demographic covariates, age, gender, race, educational status, household

income, and smoking, and clinical covariates blood pressure, body mass index (BMI), blood cholesterol level, C reactive protein (CRP) and ferritin (Tables 4, 5, 6 and 7). In addition, the PROC CROSSTAB (RTI, 2013) was used to evaluate the prevalence difference of T2DM and CVD among different categories of explanatory variables without adjusting by age, and the PROC DESCRIPT (RTI, 2013) was used to evaluate the prevalence difference of T2DM and CVD among different categories of explanatory variables adjusted by age. For all of three procedures above, the nest, weight and subpopn statements were used (Tables 2 and 3). The nest statement with strata (sdmvstra) and PSU (sdmvpsu) was used to account for the design effects. The weight statement with weight variable FASTWGT was used to account for the unequal probability of sampling and nonresponse. The subpopn statement was used to select the sample individuals 20 or older and less than 50 years ($20 \leq age < 50$) because only those individuals are of interest in this study.

The research questions are listed below again for review.

$H_1$1: Is HSV-1 infection associated with higher risk of developing T2DM?

$H_0$1: HSV-1 infection is not associated with higher risk for T2DM.

$H_1$2: Is HSV-2 infection associated with higher risk for of developing T2DM?

$H_0$2: HSV-2 infection is not associated with higher risk for T2DM.

$H_1$3: Is HSV-1 infection associated with higher risk of developing CVD?

$H_0$3: HSV-1 infection is not associated with higher risk for CVD.

$H_1$4: Is HSV-2 infection associated with higher risk of developing CVD?

$H_0$4: HSV-2 infection is not associated with higher risk for CVD.

$H_1$5: Is having a higher burden of pathogens (HSV-1 + HSV-2+CMV) associated with a higher risk of developing T2DM and/or CVD?

$H_0$5: A higher burden of pathogens is not associated with higher risk for T2DM and/or CVD.

Bias and Delimitations

Given that this study utilized data collected from a cross-sectional design, the study cannot prove causality. Only participants whose data were available between the ages of 20 and 49 with positive serology test for HSV-1 and HSV-2 and diagnosed with T2DM and CVD were included in the study. Some analyses were restricted to subpopulations for which the studied variables were collected. While data for HSV, T2DM and CVD were collected for 12 years period (1999-2010), serological status for other herpes viruses associated with inflammatory diseases such as CMV were available only for 4 years (1999-2004), which limited the opportunities for comparison and correlation analysis. In addition, data for some of the inflammatory markers were not collected consistently for the 12 years period; therefore, some analyses were restricted by this limitation.

Potential Bias

Selection Bias

NHANES selects survey participants randomly from a large cross-sectional study sample, which allows for making inferences for the general population. As a result, selection bias is not a concern.

Information Bias

Self-report bias may be a limitation of NHANES data given that the interview questions are based on information recall of the participants. Participants may provide biased information when he or she cannot recall or may modify the information when asked by the interviewer. Nevertheless, for the purpose of this study, the main variables of interest (HSV, CMV, T2DM, CVD, CRP, homocysteine, serum ferritin) were measured by laboratory and examination results rather than self-report alone. NHANES laboratory methods are conducted with high performance instruments and following strict protocols.

Confounding

A multivariate analysis was performed on variables of interest, which allows identifying potential cofounder factors.

Type I and Type II error

To control for type I error, a margin of error was set at alpha 0.05, with a probability of accuracy of 95%. Since a large sample size of 6,706 was used in the present study, type II error is not a concern.

Ethical Considerations

NHANES survey has ongoing Institutional Review Board (IRB) approval through the National Center for Health Statistics (NCHS) Research Ethics Review Board. The IRB ensures that ethical, biomedical and behavioral research best practices involving humans are in place before, during and after research studies are conducted with humans. By law, NHANES adheres to a rigorous review process in making certain that human

subject's identity is kept confidential and anonymous. In fact, all personal identifiers are removed from NHANES data prior publishing. Written consent is obtained from each survey participants. In a case of a minor, consent is obtained from parents or legal guardian. Given that I used publicly available secondary data with no link to personal identifiers, this study poses no physical or emotional risk or benefit directly or indirect to survey participants (CDC, 2012). IRB approval number for this study is 12-09-13-0075602.

Summary

This study used a cross-sectional population based designed and a large sample size from the CDC NHANES (1999-2010) dataset. Independent and dependent variables as well as covariates were selected based on a comprehensive literature review. Given the type and number of variables selected for this study, chi square was applied in univariate analysis whereas multivariate logistic and stepwise regression analyses were employed to examine the relationship between HSV and T2DM; the relationship between HSV and CVD; and the effects of burden of infections (having more than one herpes virus) on T2DM and CVD. The relationship between T2DM and CVD and selected inflammatory markers and covariates was also evaluated. Several statistical models were used to test study hypotheses of association. Adjustments for possible confounding factors were performed in all the analyses. Lastly, since this study used 12 years of data from a national representative sample, results and findings from this study can be applied to the general population.

Chapter 4: Results

Introduction

This study examined whether being HSV-1 or HSV-2 seropositive increases the risk of developing T2DM and/or CVD; and whether being seropositive to both HSVs or to three herpes viruses (HSV and CMV) increases the risk of developing T2DM and/or CVD. Further, this study evaluated whether a relationship exists among T2DM and CVD and CRP, serum ferritin, and homocysteine. It used a representative sample from participants in the United States. A cross-sectional design was used with NHANES data from 1999-2010. During that period, 99,806 participants were studied. The sample size for this study consisted of 6,706 participants, aged 20-49, who met the criteria and data from interviews, medical examination, and laboratory were available. Also evaluated were the estimates for the prevalence of each of the primary independent and dependent variables as well as for the covariates. Chapter 4 discusses the study results; important findings are presented in graphs and tables, which include the characteristics and distribution of the study population, the distribution of the herpes viruses understudy, the prevalence of T2DM and CVD in the sample, and the relationship among T2DM and CVD and HSV-2. The findings are summarized at the end of the chapter.

Demographic and Health Characteristics of Study Population

The distribution of the study population is described in Table 4. The average age was 35 years with slightly more between 35-49 years old (52.6%) than 20-34 years old (47.4%). The sample was equally divided between males (49.7%) and females (50.3%). The majority of participants were nonHispanic Whites (65.8%), followed by Hispanics

(16.9%), nonHispanic Blacks (12.5%), and other racial populations (4.9%). The participants were well educated, with more than half having earned a college degree (57.8%) and only slightly less having completed high school (42.3%). Most participants had incomes well above poverty, with nearly half earning annually more than $55,000 (47.0%) and about one-third between $25,000-54,000 (31.9%). The majority of participants were nonsmokers (55.2 %). Clinical data indicated that nearly all of the participants (91.8%) were not hypertensive; however, only 36.9% had a normal BMI (less than 25). The rest of the participants were either overweight (32.4% with a BMI 25-29) or obese (30.8 % with a BMI $\geq$ 30). Nearly all participants had low-normal CRP levels (90.5 %). Overall, ferritin levels for participants were normal with the majority of men (83.3 %) and women (94.7%) in the low to normal range. Most participants had normal (97.3%) homocysteine levels and a few (2.7%) had moderate levels. Since no participants had elevated levels of homocysteine, this marker was excluded from association analyses. More than half of the participants had cholesterol levels that were desirable (61.0%); about one-quarter had borderline (26.9%) and only 12.0 % had high cholesterol levels.

Table 4

Demographic and Health Characteristics, NHANES, 1999-2010

Characteristics	n^{α}	Percentage (95% CI)
Total Population	6706	
Age Mean		34.92 [34.58,35.26]
Gender		
Male	3106	49.68 [48.63,50.73]
Female	3600	50.32 [49.27,51.37]
Race/Ethnicity		
NonHispanic White	2931	65.80 [63.06,68.44]
NonHispanic Black	1376	12.47 [11.00,14.12]
Hispanic	1958	16.85 [14.84,19.06]
Other	441	4.88 [3.98,5.98]
Age (years)		
20~34	3401	47.37 [45.49,49.27]
35~49	3305	52.63 [50.73,54.51]
Education		
High School	3325	42.25 [39.94,44.60]
College or higher	3373	57.75 [55.40,60.06]
Household Income		
<25,000	1014	21.11 [18.99,23.40]
25,000~54,000	1347	31.85 [29.36,34.45]
>55,000	1522	47.04 [44.16,49.94]
Smoking Status		
Nonsmoker	3884	55.23 [53.17,57.28]
Smoker	2817	44.77 [42.72,46.84]
Hypertension		
Yes	502	8.25 [7.54,9.03]
No	5360	91.75 [90.97,92.47]
BMI(kg/m^2)		
<25 (normal)	2223	36.87 [35.42,38.35]
25~29 (overweight)	2213	32.37 [30.87,33.92]
≥30 (obese)	2201	30.76 [29.25,32.30]
CRP Levels		
Low-Normal	5949	90.50[89.77,91.19]
Average	651	8.32 [7.62,9.08]
High	80	1.18 [0.88,1.58]
Ferritin Levels (Men)		
Low	9	1.05 [0.47,2.32]

Normal	881	82.25 [79.23,84.92]
High	160	16.69 [14.16,19.58]
Ferritin Levels (Women)		
Low	779	20.28 [18.69,21.96]
Normal	2133	74.46 [72.46,76.37]
High	140	5.26 [4.30,6.43]
Homocysteine Levels[b]		
Normal	2003	97.33 [96.28,98.09]
Moderate	46	2.67 [1.91,3.72]
Elevated	0	
Cholesterol Levels		
< 200 (normal)	3936	61.03 [59.60, 62.44]
200~239 (borderline)	1849	26.93 [25.65,28.26]
≥240 (high)	870	12.04[11.00,13.15]

[α] Data are missing for all variables except for age, gender, and race/ethnicity.
[b] Data available only for 1999-2006, homocysteine was excluded from association analyses.

Distribution of Herpes Viruses

Overall, a decline in all three herpes viruses (HSV-1, HSV-2, CMV) was observed in this sample population from 1999 (HSV 62.4%, 23.7%) to 2010 (HSV 54.2%, 17.2%) respectively (Figure 4). A decline was also observed in CMV from 1999 (56.7%) to 2004 (53.0%). A similar trend was observed among the participants who were seropositive to both, HSV-1and HSV-2 (14.8% in 1999) to (10.6% in 2010) and among the participants who were positive to HSV and CMV (11.3% in 1999) to (8.5% in 2004).

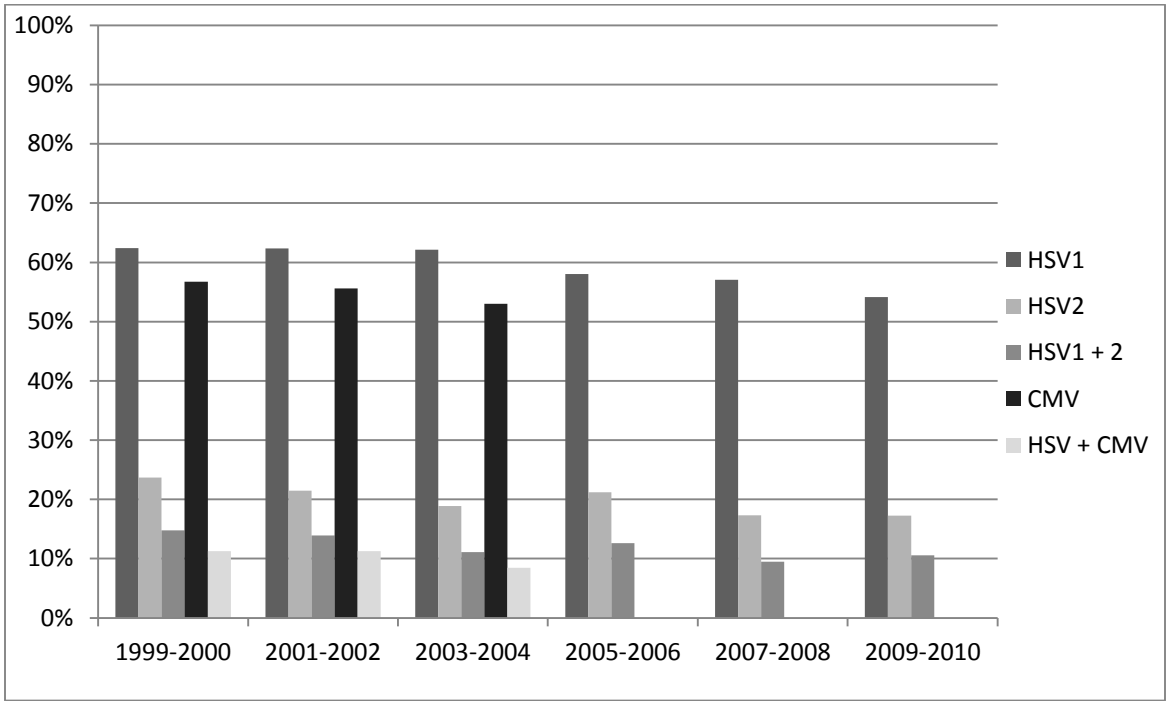

Figure 4. Percentage of participants seropositive for HSV-1, HSV-2, HSV-1+HSV-2, CMV, and all three herpes from 1999-2010 in this U.S. population sample. Data for CMV and burden of infection (three herpes viruses HSV and CMV) is not available after 2004.

In this study, more females than males were seropositive to all herpes virus infections, with females having almost double the number of HSV-2 infections, 990 (26.4%) vs 499 (13.45) and HSV-1and HSV-2 infections, 669 (16.5%) vs 309 (7.55) than males (Figure 5). A slightly difference was observed in the proportions of HSV-1 and CMV among females, 2,391 (61.7%) vs 1,144 (62.1%) and among males, 1,932 (56.9%) vs 823 (47.9%) respectively.

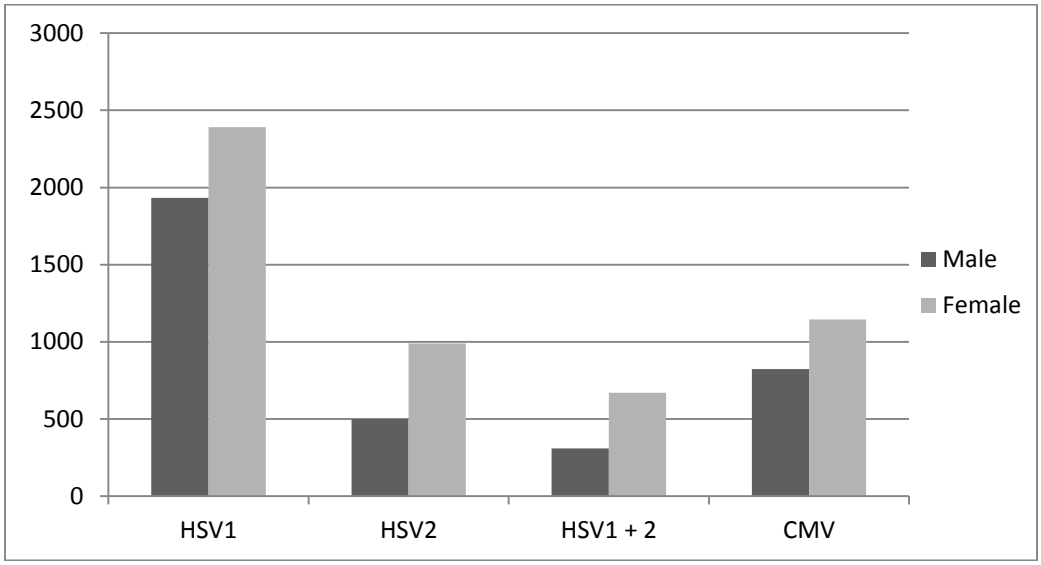

Figure 5. Number of participants seropositive for HSV-1, HSV-2, HSV-1and HSV-2, and CMV by gender from 1999-2010. CMV data only available from 1999-2004.

Results of this study indicated that CMV and HSV-1 were predominantly high across all racial groups (Figure 6). Particularly, HSV-1 was high among Hispanics (79.2%), followed by other race groups (77.9%), nonHispanic Blacks, and nonHispanic Whites (51.8%). Similarly, CMV was higher among other ethnic populations (84.6%) and Hispanics (80.9%), followed by nonHispanic Blacks (77.7%). CMV was far less common among nonHispanic Whites (43.0%). In comparison to all racial groups, a higher proportion of nonHispanic Blacks were seropositive to HSV-2 (46.7%) and HSV-1and HSV-2 (30.0%), followed by other racial groups (HSV-2, 29.2%) and (HSV-1+HSV-2, 23.4%).

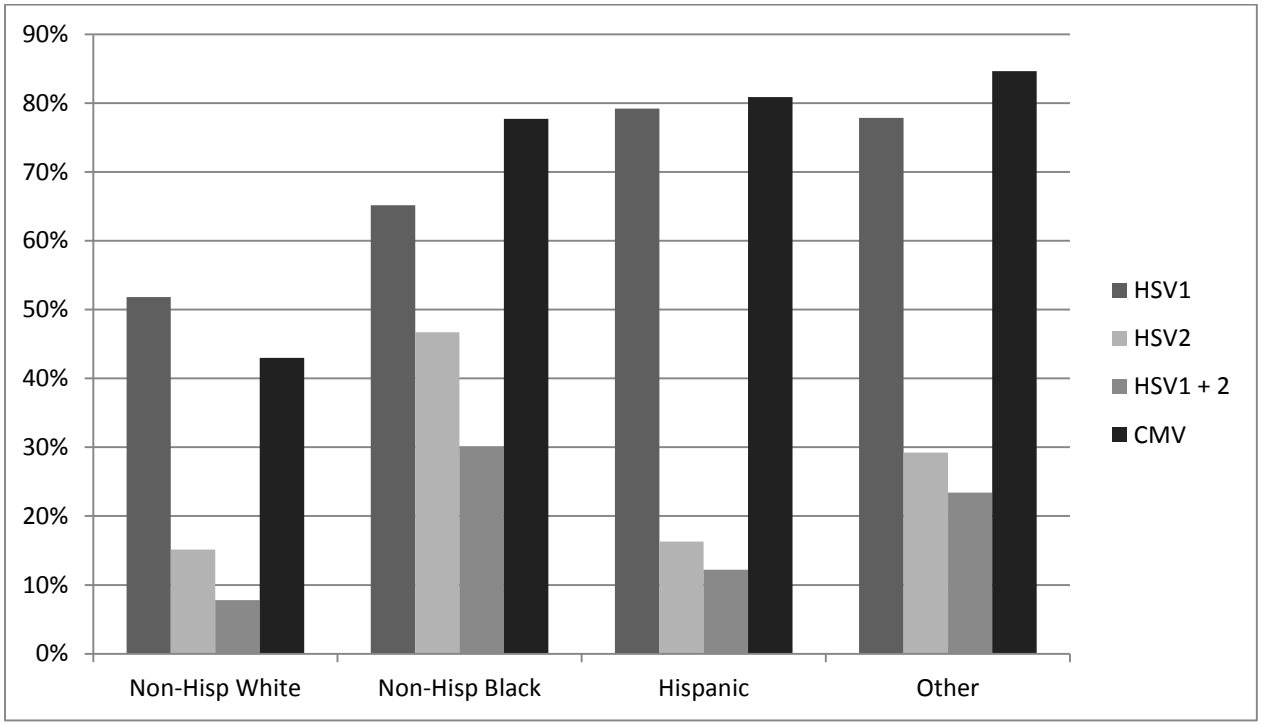

Figure 6. Percentage of participants seropositive for HSV-1, HSV-2 and CMV by ethnicity from 1999-2010. CMV data only available from 1999-2004.

All herpes virus infections were higher among individuals between the ages of 35-49, with this group having almost twice the number of HSV-2, 976 (26.3%) vs 513(12.9%) and over twice the number of HSV-1and HSV-2, 674(16.6%) vs 304(7.0%) (Figure 7). A slightly difference was observed between individuals aged 20-35 and 35-49 that were seropositive to HSV-1, 2,027 (53.3%) vs 2,296 (64.8%) and CMV, 954 (51.3%) vs 1013 (58.3%) respectively.

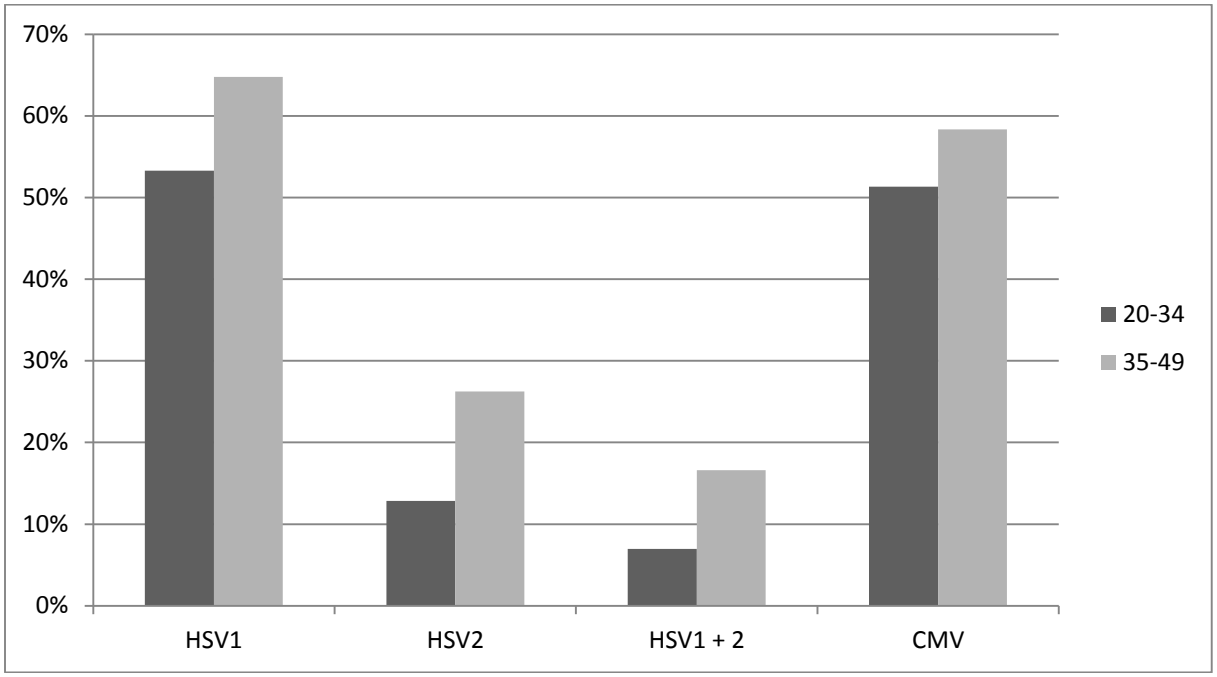

Figure 7. Percentage of participants seropositive for HSV-1, HSV-2 and CMV by age groups from 1999-2010. CMV data only available from 1999-2004.

Prevalence of T2DM and CVD by Selected Health Characteristics

The prevalence of T2DM and CVD among selected characteristics in the study sample was examined using Pearson chi-square test (Figure 5). An elevated prevalence of T2DM was observed in gender, race/ethnicity, age, household income, hypertension, and BMI. Particularly, the age-adjusted prevalence of T2DM was significantly higher in males 5.13% [95% CI=4.14, 6.35] than in females 3.70% [95% CI = 2.97,4.60]; significantly higher among Hispanics 6.62% [95% CI = 5.03, 8.66], followed by other racial groups, 6.22% [95% CI = 3.83, 9.95]; and nonHispanic Blacks 6.12% [95% CI=4.89, 7.64] compared to nonHispanic Whites, 3.51% [95% CI = 2.74,4.49]. T2DM was over three times higher in individuals aged 35-49, 6.63% [95% CI = 5.53, 7.93] than among aged 20-34, 1.78% [95% CI = 1.36, 2.34]; significantly higher among individuals

with a household income between 25,000 and 54,999, 6.22% [95% CI = 4.53,8.48], and with an income less than 25,000 6.06% [95% C I= 4.34, 8.39] than among individuals with income ≥55,000; and more than two fold higher among individuals with hypertension 9.44 [95% CI = 6.46, 13.6] than those without hypertension 3.72% [95% CI=3.08,4.49]. Higher prevalence was found among obese individuals with a BMI ≥30 8.80% [95% CI = 7.36, 10.5]; followed by overweight individuals with a BMI 25-29 2.91% [95% CI = 2.14, 3.95] than among individuals with a BMI ≤ 25. However, no difference was observed in the prevalence of T2DM and education, smoking status, or cholesterol levels.

Similarly, the age-adjusted prevalence of CVD was found higher among individuals aged 35-49, 3.60% [95% CI = 2.89,4.48] than among the younger group; with a household income less than 25,000, 3.82% [95% CI = 2.40,6.02]; followed by individuals with a household income between 25,000-54,999, 2.92% [95% CI=1.99,4.25] than among individuals with an income ≥ 55,000; with hypertension 3.66% [95% CI=2.31, 5.76] than among individuals without hypertension; among obese individuals (BMI ≥30), 3.39% [95% CI = 2.58, 4.44], followed by individuals overweight (BMI between 25-29) 2.12% [95% CI = 1.49, 2.99] than among individuals with a BMI ≤25. In contrast to T2DM, CVD was significantly higher among individuals with a high school education 3.00% [95% CI = 2.35, 3.84] compared to those with a college or higher degree 1.80% [95% CI = 1.31, 2.48]; and among individuals that smoke or are past smokers 3.65% [95% CI = 2.90, 4.59] in comparison to those that never smoke. The

prevalence of CVD was not significantly different by gender, race/ethnicity, or cholesterol level.

Table 5

Prevalence (%) of Reported T2DM and CVD among U.S. 20 to 49 Years Old by Selected Characteristics, NHANES, 1999-2010

Characteristics	T2DM Unadjusted (95%CI)	T2DM Age-Adjusted (95% CI)	CVD Unadjusted (95%CI)	CVD Age-Adjusted (95% CI)
Gender	**	**		
Male	5.01 [4.03,6.20]	5.13[4.14,6.35]	2.12[1.64,2.75]	2.19[1.68,2.83]
Female	3.67[2.95,4.57]	3.70[2.97,4.60]	2.39[1.78,3.21]	2.41[1.79,3.24]
Race/Ethnicity	***	***		
NonHispanic White	3.57[2.79,4.56]	3.51[2.74,4.49]	2.39[1.82,3.13]	2.34[1.78,3.07]
NonHispanic Black	5.90[4.72,7.36]	6.12[4.89,7.64]	2.79[2.01,3.84]	2.93[2.15,3.99]
Hispanic	5.72[4.28,7.61]	6.62[5.03,8.66]	1.60[1.10,2.32]	1.73[1.18,2.52]
Other	5.94[3.63,9.59]	6.22[3.83,9.95]	1.46[0.61,3.46]	1.59[0.66,3.78]
Age (years)	***	***	***	***
20~34	1.78[1.36,2.34]	1.78[1.36,2.34]	0.77[0.51,1.17]	0.77[0.51,1.17]
35~49	6.63[5.53,7.93]	6.63[5.53,7.93]	3.60[2.90,4.48]	3.60[2.89,4.48]
Education			**	**
High School	4.62[3.78,5.62]	4.79[3.96,5.79]	2.89[2.26,3.69]	3.00[2.35,3.84]
College or higher	4.14[3.29,5.20]	4.14[3.28,5.21]	1.80[1.31,2.48]	1.80[1.31,2.48]
Household Income	**	**	***	***
<25,000	5.48[3.85,7.73]	6.06[4.34,8.39]	3.43[2.16,5.43]	3.82[2.40,6.02]
25,000,54,000	5.81[4.25,7.89]	6.22[4.53,8.48]	2.68[1.82,3.92]	2.92[1.99,4.25]
>55,000	2.98[1.97,4.50]	2.77[1.81,4.22]	1.56[1.00,2.46]	1.45[0.92,2.27]
Smoking Status			***	***
Never smoke	4.01[3.30,4.87]	4.20[3.44,5.11]	1.05[0.74,1.50]	1.10[0.77,1.56]
Past or current Smoke	4.75[3.84,5.85]	4.64[3.75,5.72]	3.75[2.98,4.72]	3.65[2.90,4.59]
Hypertension	***	***	**	**
Yes	10.48[7.60,14.28]	9.44[6.46,13.6]	4.79[3.01,7.54]	3.66[2.31,5.76]
No	3.59[2.97,4.33]	3.72[3.08,4.49]	2.03[1.57,2.61]	2.11[1.63,2.71]
BMI(kg/m^2)	***	***	***	***
<25	1.31[0.87,1.98]	1.46[0.96,2.20]	1.11[0.70,1.75]	1.21[0.75,1.94]
25,29	2.96[2.18,4.01]	2.91[2.14,3.95]	2.16[1.52,3.06]	2.12[1.49,2.99]

≥30	9.08[7.60,10.83]	8.80[7.36,10.5]	3.52[2.67,4.62]	3.39[2.58,4.44]
Cholesterol Levels				
< 200	4.04[3.32,4.91]	4.52[3.70,5.50]	2.09[1.63,2.68]	2.34[1.83,2.99]
200-239	4.41[3.23,5.99]	4.13[3.04,5.60]	2.23[1.53,3.24]	2.07[1.42,3.00]
≥240	5.28 [3.79,7.31]	4.73[3.38,6.59]	2.96[1.76,4.93]	2.31[1.37,3.87]

*$p < 0.10$, ** $p < 0.05$, ***$p < 0.01$

The prevalence of T2DM and CVD by each of the herpes virus infections and selected inflammatory markers is illustrated (Table 6). Results indicated that the prevalence of T2DM and CVD was significantly higher among individuals seropositive to HSV-2, 5.54% [95% CI = 4.36, 7.01] and 3.32% [95% CI = 2.36, 4.65] respectively, but not to HSV-1 or CMV. Age-adjusted prevalence of T2DM was significantly elevated among individuals with average to high CRP levels 10.9% [95% CI = 8.11, 14.5] than individuals with low-normal levels 3.63% [95% C I= 3.05, 4.33]; among males with high ferritin levels 7.85% [95% CI = 3.68, 15.96] than males with normal levels 5.32% [95% CI = 3.34, 8.37] and among females with high levels 12.6% (95% CI = 6.11, 24.07). There was no data for low ferritin levels among males. Further, the prevalence of CVD was higher among females with high 4.85% [95% C I= 2.53, 9.09] ferritin levels. No relationship was observed between the prevalence of CVD and CRP, male's ferritin levels, or homocysteine neither between T2DM and homocysteine levels. There was no data of elevated homocysteine levels for T2DM or CVD; therefore, homocysteine was excluded from logistic regression analyses.

Table 6

Prevalence (%) of T2DM and CVD Among U.S. 20 to 49 Years Old by Herpes Viruses Infections and Selected Inflammatory Markers, NHANES

Characteristics	T2DM Unadjusted (95%CI)	T2DM Age-adjusted (95% CI)	CVD Unadjusted (95%CI)	CVD Age-Adjusted (95% CI)
HSV-1				
Yes	4.56[3.79,5.48]	4.40[3.66,5.29]	2.44[1.90,3.11]	2.36[1.85,3.01]
No	3.86[2.96,5.02]	4.26[3.24,5.57]	2.01[1.47,2.75]	2.29[1.67,3.15]
HSV-2	***	***	***	***
Yes	6.15[4.87,7.75]	5.54[4.36,7.01]	3.96[2.82,5.52]	3.32[2.36,4.65]
No	3.80[3.13,4.62]	4.06[3.32,4.96]	1.85[1.46,2.33]	1.97[1.56,2.49]
CMV [a]				
Yes	4.30[3.08,6.00]	4.16[3.03,5.70]	1.97[1.31,2.96]	1.92[1.28,2.85]
No	4.27[2.97,6.09]	4.47[3.08,6.45]	1.90[1.13,3.19]	2.05[1.20,3.49]
CRP	***	***		
Low-Normal	3.56[2.99,4.24]	3.63[3.05,4.33]	2.07[1.65,2.59]	2.11[1.69,2.64]
Average to High	11.1[8.21,11.9]	10.9[8.11,14.5]	3.84[2.44,6.00]	3.74[2.41,5.76]
Ferritin Levels (Males)	**	**		
Low	0.00	0.00	0.00	0.00
Normal	5.11[3.23,8.00]	5.32[3.34,8.37]	2.32[1.32,4.03]	2.39[1.36,4.19]
High	8.93[4.06,18.52]	7.85[3.68,15.96]	3.67[1.53,8.53]	2.90[1.18,6.93]
Ferritin Levels (Females)	***	***	*	*
Low	2.36[1.55,3.57]	2.49[1.62,3.80]	1.72[0.82,3.57]	1.77[0.84,3.70]
Normal	3.32[2.47,4.45]	3.38[2.53,4.51]	2.26[1.52,3.34]	2.32[1.56,3.44]
High	12.15[7.08,20.08]	12.56[6.11,24.07]	6.34[3.30,11.85]	4.85[2.53,9.09]
Homocysteine Levels[b]				
Normal	4.05[2.94,5.56]	4.10[2.99,5.62]	2.81[1.94,4.07]	2.88[1.97,4.17]
Moderate	7.42[3.04,17.04]	5.74[2.24,13.91]	5.66[1.56,18.54]	6.66[1.63,23.50]
Elevated	0.00	0.00	0.00	0.00

*$p < 0.10$, ** $p < 0.05$, ***$p < 0.01$

[a] Data available only for 1999-2004

[b] Data available only for 1999-2006

Relationship Between T2DM and HSV

The relationship between T2DM and HSV-2 as well as demographic and clinical variables was explored (Table 7). Results from the logistic regression analysis (crude odds ratios) showed that T2DM was associated with HSV-2, age, gender, race, household income, blood pressure, BMI, CRP, and ferritin. Specifically, T2DM was 1.66 times more likely to occur among individuals who were HSV-2 positive than among individuals who were not ($p = 0.0010$) if not adjusted by any factor (Column 1 Table 7). The odd ratio between T2DM and HSV-2 adjusted for each of demographic and clinical variables were also calculated (Column 2, Table 7). T2DM was 1.81 times more likely to occur among individuals who were HSV-2 positive than among individuals who were not if adjusted by gender ($p = 0.0001$); 1.52 adjusted by race ($p = 0.0048$); 1.65 ($p = 0.0011$) adjusted by education; 1.63 ($p = 0.0012$) adjusted by smoking; 1.67 ($p = 0.0003$) adjusted by blood pressure; 1.48 ($p = 0.0150$) adjusted by BMI; 1.64 ($p = 0.0014$) adjusted by cholesterol level; 1.53 ($p = 0.0054$) adjusted by CRP; and 2.11 if adjusted by ferritin ($p = 0.0008$). Based on the confounding 10% rule (Vittinghoff, Glidden, Shiboski, & McCulloch, 2012), it was observed that gender and ferritin were positive confounders, while age, income and BMI were negative confounders of HSV-2.

To examine the predictive effects of multiple variables simultaneously, a stepwise regression was performed (Columns 3 and 4, Table 7). After adjusting for all potential predictive demographic variables for T2DM (age, gender, household income, BMI, CRP, and ferritin), the association between T2DM and HSV-2 was no longer significant. However, all confounding factors included in the regression model (Table 7) were

significant at either alpha=0.10, 0.05, or 0.01. The presence of confounding implies that the adjusted summary measure is a better estimate of the effect of the exposure on the outcome of interest since it removes the effect of all possible confounders. Therefore, model 4 (column 4, tables 7) provides the best effect estimate and confirms the null results.

Table 7

Odds Ratios (95% Confidence Intervals) for T2DM Associated With HSV-2 by Selected Characteristics Among U.S. 20 to 49 Years Old Population, NHANES, 1999-2010

	(1) Crude[a]	(2) Adjusted[b]	(3) Adjusted[c]	(4) Adjusted[d]
Ref (negative)	1.00	1.00	1.00	1.00
HSV-2 (positive)	1.66[1.23,2.23]***	1.66[1.23,2.23]***	1.02[0.67,1.53]	1.50[0.85,2.65]
Age Group (35~49 vs 20~34)	3.91[2.85,5.36]***	1.33[0.97,1.83]*	4.22[2.62,6.80]***	3.03[1.61,5.71]***
Gender (Male vs Female)	1.38[1.02,1.88]**	1.81[1.36,2.41]***	1.43[0.93,2.22]	1.70[0.92,3.13]*
Race (nonWhite vs White)	1.67[1.23,2.27]***	1.52[1.14,2.03]***	-	-
Education (high school vs college or higher)	1.12[0.83,1.51]	1.65[1.23,2.21]***	-	-
Household Income		1.24[0.81,1.88]		
<$25,000 vs >$55,000	1.88[1.06,3.35]**		2.48[1.36,4.54]***	2.12[0.91,4.93]*
$25,000~54,999 vs >$55,000	2.01[1.16,3.47]**		2.37[1.32,4.24]***	2.29[0.98,5.38]*
Smoking (Smoker vs nonsmoker)	1.19[0.92,1.54]	1.63[1.22,2.19]***	-	-
Blood Pressure (High vs Normal)	3.14[2.15,4.59]***	1.67[1.27,2.20]***	-	-
BMI		1.48[1.08,2.03]**		
Overweight vs Normal	2.30[1.44,3.66]***		-	2.45[1.09,5.52]**
Obesity vs Normal	7.53[4.70,12.1]***		-	6.91[2.56,18.7]***
Blood Cholesterol Level		1.64[1.22,2.21]***		
Borderline high vs Desirable	1.10[0.77,1.55]		-	-
High levels vs Desirable	1.32[0.88,2.00]		-	-
C reactive protein		1.53[1.14,2.07]***		

High or Average vs Low or normal	3.39[2.37,4.84]***		-	2.22[1.16,4.27]**
Ferritin		2.11[1.38,3.24]***		
Normal levels vs Low levels	1.70[1.01,2.86]**		-	2.10[0.84,5.23]
High levels vs Low levels	4.89[2.55,9.38]***		-	4.42[1.52,12.8]***

--: the predictor not included in the final multiple logistic regression model, * $p < 0.10$, ** $p < 0.05$, ***$p < 0.01$

[a]: odds ratio (95% CI) btw T2DM by each risk factor only.

[b]: odds ratio (95% CI) btw T2DM and HSV-2 adjusted by each risk factor.

[c]: odds ratio (95% CI) btw T2DM and HSV-2 adjusted by demographic variables in one full model.

[d]: odds ratio (95% CI) btw T2DM and HSV-2 adjusted by demographic and clinical variables in one full model.

Similarly, the correlation between T2DM and the number of infections with HSV-1+HSV-2 was also examined (Table 8). The results of this analysis were consistent with those described in table 7. T2DM was associated with HSV-2, age, gender, race, household income, blood pressure, BMI, and ferritin. Unadjusted rates indicate that T2DM was 1.99 times more likely to occur among individuals who were seropositive for both HSV-1and HSV-2 than among individuals who acquired neither one ($p = 0.0042$). T2DM was 3.91 times more likely to occur among individuals aged 35~49 than among individuals aged 20~34 ($p < 0.0001$); 1.38 times more likely to occur among male than among female ($p = 0.0392$); 1.67 times more likely to occur among nonWhites than among Whites ($p = 0.0013$); 1.88 times more likely to occur among individuals with a household income below $25,000 than among individuals with a household income between $25,000 and $49,999 ($p = 0.0309$); 2.01 times more likely to occur among individuals with household income above $25,000 and less than $55,000 than among individuals with household income above $55,000 ($p = 0.0133$); 3.14 times more likely to occur among individuals with high blood pressure than among individuals with normal

blood pressure ($p < 0.0001$); 2.30 times more likely to occur among overweight individuals than among normal weight individuals ($p = 0.0006$) ; 7.53 times more likely to occur among obese individuals than among normal weight ($p < 0.0001$); 3.39 times more likely to occur among individuals with high to average level CRP than among individuals with low to normal CRP ($p < 0.0001$); 1.70 times more likely to occur among individuals with normal ferritin levels than among individuals with low levels (p=0.0461); and 4.89 more likely to occur among individuals with high levels of ferritin than among those with low levels ($p < 0.0001$) . However, T2DM was not associated with HSV-1, education, smoking, and blood cholesterol level.

Adjusted rates determined that T2DM was 2.22 times more likely to occur among individuals who were seropositive for HSV-1and HSV-2 than among individuals who were not ($p = 0.0004$) adjusted by gender (Column 2, Table 8); 1.69 ($p = 0.0355$) adjusted by race; 1.98 ($p = 0.0042$; adjusted by education; 1.96 ($p = 0.0051$) adjusted by smoking; 2.09 ($p = 0.0021$) adjusted by blood pressure; 1.71 ($p = 0.0287$) adjusted by BMI; 1.96 ($p = 0.0057$) adjusted by blood cholesterol; 1.82 ($p = 0.0152$) adjusted by CRP; and 2.29 ($p = 0.0246$) if adjusted by ferritin. Having HSV-1and HSV-2 versus not having acquired any herpes virus was not significantly associated with T2DM when adjusted by age, gender, household income, BMI, CRP, or ferritin. However, all these confounders exhibited significance in this model (Column 3 and 4, Table 8).

Table 8

Odds Ratios (95% Confidence Intervals) for T2DM Associated With Herpes Simplex Viruses (HSV-1and HSV-2) by Selected Covariates Among U.S. 20 to 49 Years Old Population, NHANES, 1999-2010

	(1) Crude[a]	(2) Adjusted[b]	(3) Adjusted[c]	(4) Adjusted[d]
Herpes Type				
One type vs no herpes (HSV-1)	1.20[0.78,1.85]	1.20[0.78,1.85]	1.02[0.57,1.83]	0.82[0.37,1.81]
Two types vs no herpes (HSV-1 and HSV-2)	1.99[1.25,3.18]***	1.99[1.25,3.18]***	1.10[0.56,2.16]	1.50[0.62,3.67]
Age Group (35~49 vs 20~34)	3.91[2.85,5.36]***	1.02[0.66,1.59]	4.17[2.53,6.88]***	3.01[1.58,5.73]***
		1.42[0.88,2.32]		
Gender (Male vs Female)	1.38[1.02,1.88]**	1.23[0.81,1.89]	1.44[0.94,2.20]*	1.69[0.94,3.03]*
		2.22[1.44,3.43]***		
Race (nonWhite vs White)	1.67[1.23,2.27]***	1.10[0.70,1.71]	-	-
		1.69[1.04,2.74]**		
Education (high school vs college or higher)	1.12[0.83,1.51]	1.20[0.77,1.85]	-	-
		1.98[1.24,3.16]***		
Household Income		1.23[0.68,2.22]		
		1.56[0.77,3.15]		
<$25,000 vs >$55,000	1.88[1.06,3.35]**		2.45[1.32,4.54]***	2.09[0.90,4.84]*
$25,000~54,999 vs >$55,000	2.01[1.16,3.47]**		2.34[1.31,4.18]***	2.28[1.00,5.19]*
Smoking (Smoker vs nonsmoker)	1.19[0.92,1.54]	1.20[0.78,1.84]		-
		1.96[1.23,3.12]***		
Blood Pressure (High vs Normal)	3.14[2.15,4.59]***	1.34[0.85,2.10]	-	
		2.09[1.32,3.33]***		
BMI		1.10[0.70,1.71]		
		1.71[1.06,2.77]**		
Overweight vs Normal	2.30[1.44,3.66]***		-	2.44[1.09,5.46]**
Obesity vs Normal	7.53[4.70,12.08]***		-	6.91[2.53,18.87]***
Blood Cholesterol Level		1.19[0.77,1.84]		
		1.96[1.22,3.15]***		
Borderline high vs Desirable	1.10[0.77,1.55]		-	-

High levels vs Desirable	1.32[0.88,2.00]		-	-
C reactive protein		1.17[0.75,1.83] [1.82,1.13 2.93]**		
High or Average vs Low or Normal	3.39[2.37,4.84]***		-	2.21[1.15,4.22]**
Ferritin		1.06[0.54,2.11] 2.29[1.11,4.70]**		
Normal levels vs Low levels	1.70[1.01,2.86]**		-	2.10[0.85,5.22]
High levels vs Low levels	4.89[2.55,9.38]***		-	4.52[1.54,13.25]***

--: the predictor not included in the final multiple logistic regression model, *$p < 0.10$, ** $p < 0.05$, ***$p<0.01$

[a]: odds ratio (95% CI) btw T2DM and each risk factor only.

[b]: odds ratio (95% CI) btw T2DM and HSV adjusted by each risk factor.

[c]: odds ratio (95% CI) btw T2DM and HSV, demographic variables, and smoke in one full model.

[d]: odds ratio (95% CI) btw T2DM and HSV, demographic variables, smoke and all clinical variables in one full model.

Relationship between CVD and HSV-2 and Burden of Infection

The interaction between CVD and HSV-2 and selected demographic and clinical variables was also evaluated (Table 9). Similar to T2DM, results of the multiple logistic analysis (Column 1, Table 9 & 10) indicated that CVD was associated with individuals who were seropositive for HSV-2, age, education, household income, smoking, blood pressure, BMI, CRP, and ferritin. Specifically, CVD was 2.19 times more likely to occur among individuals who were HSV-2 positive than among individuals who did not (p = 0.0002) and 2.65 (Column 1, Table 10) times more likely to occur among individuals who acquired both HSV-1and HSV-2 than among individuals who acquired neither one (p = 0.0013); 4.81 times as likely to occur among individuals aged 35~49 than among individuals aged 20~34 (p <0.0001); 1.62 times more likely to occur among individuals with high school education than among individuals with college or higher education (p = 0.0192); 2.24 times more likely to occur among individuals with household income

below $25,000 than among individuals with household income above $25,000 and less than $55,000 ($p = 0.0040$); 1.73 times more likely to occur among individuals with household income above $25,000 and less than $55,000 than among individuals with household income above $55,000 ($p = 0.0609$); 3.66 times more likely to occur among smoking individuals than among nonsmoking individuals ($p < 0.0001$); 2.43 times more likely to occur among individuals with high blood pressure than among individuals with normal blood pressure ($p = 0.0017$); 1.97 times more likely to occur among overweight individuals than among normal weight individuals ($p = 0.0185$); 3.25 times more likely to occur among obese individuals than among normal weight ($p < 0.0001$); 1.89 times more likely to occur among individuals with high to average level CRP than among individuals with low to normal CRP ($p = 0.0130$); 2.97 times more likely to occur among individuals with high level ferritin than among individuals with low level ferritin ($p = 0.0185$). Nonetheless, CVD was not associated with HSV-1, CMV, gender, race, cholesterol level, or burden of infections.

The odds ratios between CVD and HSV-2 adjusted by each of the demographic and clinical variables are shown (Column 2, Table 9 &10). CVD was found 1.73 ($p=0.0097$) times more likely to occur among individuals who were seropositive for HSV-2 than among individuals who were not if adjusted by age (Column 2, Table 9); 2.21($p = 0.0001$) adjusted by gender; 2.32 ($p = 0.0001$) adjusted by race; 2.13($p=0.0003$) adjusted by education; 1.91 ($p = 0.0024$) adjusted by smoking; 2.06 ($p = 0.0011$) adjusted by blood pressure; 2.07 ($p = 0.0009$) adjusted by BMI; 2.16 ($p = 0.0003$) adjusted by blood cholesterol; 2.12 ($p = 0.0004$) adjusted by CRP; and 2.07 ($p = 0.0023$) adjusted by

ferritin. Based on the 10% rule (Vittinghoff et al., 2012), it was observed that age, household income and smoking were negative confounders of HSV-2. To identify potential confounders between CVD and HSV-2, a stepwise regression analysis was also performed. CVD was not associated with HSV-2 when adjusted by age, household income; and smoking and BMI (Column 3, Table 9).Nevertheless, all the confounding variables were found significant at alpha=0.10, 0.05, or 0.01 (Column 4, Tables 9 & 10).

Table 9

Odds Ratios (95% Confidence Intervals) for CVD Associated With HSV-2 and Burden of Infection by Selected Covariates Among U.S. 20 to 49 Years Old Population, NHANES, 1999-2010

	(1) Crude[a]	(2) Adjusted[b]	(3) Adjusted[c]	(4) Adjusted[d]
HSV-2 (Yes vs No)	2.19[1.47,3.27]***	2.19[1.47,3.27]***	1.03[0.59,1.80]	1.08[0.59,1.95]
BURDEN*		-		-
1 vs 0	1.03[0.48,2.18]		-	-
2 vs 0	0.69[0.28,1.68]		-	-
3 vs 0	1.62[0.72,3.66]		-	
Age Group (35~49 vs 20~34)	4.81[3.04,7.63]***	1.73[1.15,2.62]***	5.19[2.59,10.37]***	4.48[2.25,8.94]***
Gender (Male vs Female)	0.89[0.60,1.31]	2.21[1.50,3.25]***	-	-
Race (nonWhite vs White)	0.84[0.58,1.22]	2.32[1.53,3.52]***	-	-
Education (high school vs college or higher)	1.62[1.08,2.42]**	2.13[1.42,3.18]***	-	-
Household Income		1.50[0.88,2.55]		
<$25,000 vs >$55,000	2.24[1.30,3.86]***		2.45[1.39,4.32]***	2.34[1.33,4.10]***
$25,000~54,999 vs >$55,000	1.73[0.97,3.08]*		1.92[1.06,3.48]**	1.80[0.94,3.42]*
Smoking (Smoker vs nonsmoker)	3.66[2.42,5.53]***	1.91[1.26,2.87]***	2.47[1.39,4.41]***	2.44[1.40,4.27]***
Blood Pressure (High vs Normal)	2.43[1.41,4.20]***	2.06[1.35,3.16]**	-	-
BMI		2.07[1.36,3.15]***		
Overweight vs Normal	1.97[1.12,3.46]**		-	3.18[1.45,6.97]***
Obesity vs Normal	3.25[1.89,5.62]***		-	3.67[1.70,7.93]***
CMVIGG	1.04[0.60,1.79]	-	-	-
Blood Cholesterol Level		2.16[1.44,3.24]***		
Borderline high vs Desirable	1.07[0.69,1.66]		-	-
High levels vs Desirable	1.43[0.78,2.60]		-	-
C reactive protein		2.12[1.41,3.18]***		
High or Average vs Low or Normal	1.89[1.12,3.20]**		-	-
Ferritin		2.07[1.30,3.27]**		
Normal vs Low	1.36[0.61,3.04]		-	-
High vs Low	2.97[1.21,7.34]**		-	-

--: the predictor not included in the final multiple logistic regression model, *$p < 0.10$, ** $p < 0.05$, ***$p<0.01$ [a]: odds ratio (95% CI) btw CVD by each risk factor only.
[b]: odds ratio (95% CI) btw CVD and HSV-2 adjusted by each risk factor.

c: odds ratio (95% CI) btw CVD and HSV-2, demographic variables in one full model.
d: odds ratio (95% CI) btw CVD and HSV-2, demographic variables, and clinical and biometric variables in one full model.
*: number of herpes infections (HSV-1 or HSV-2, HSV-1 and HSV-2, and HSV-1 and HSV-2 and (CMV)

Table 10 illustrates the odd ratio for CVD and HSV-1 and HSV-2 and selected markers. Study results indicated that CVD was 1.84 times more likely to occur among individuals who were seropositive to both, HSV-1 and HSV-2 than among individuals who were not ($p = 0.0524$) if adjusted by age; 2.66 ($p = 0.0006$) adjusted by gender; 3.02 ($p=0.0008$) adjusted by race; 2.40 ($p = 0.0051$) adjusted by education; 2.20 ($p = 0.0094$) adjusted by smoking; 2.72 ($p = 0.0012$) adjusted by blood pressure; 2.45 ($p =0.0062$) adjusted by BMI; 2.60 ($p = 0.0015$) adjusted by blood cholesterol level; 2.54 ($p = 0.0020$) adjusted by CRP; 2.63 ($p = 0.0056$) adjusted by ferritin. The predictive effects of multiple variables were also assessed with a stepwise regression (3^{rd} and 4^{th} columns, Table 10). Results were comparable with T2DM, indicating not significant association between CVD and having HSV-1 and HSV-2 if adjusted by age, household income and smoking; however, age, household income and smoking showed significance in all regression models.

Table 10

Odds Ratios (95% Confidence Intervals) for CVD Associated With HSV-1 and HSV-2 and Selected Markers Among U.S. 20 to 49 Years Old Population, NHANES, 1999-2010 (HSV)

	(1) Crude[a]	(2) Adjusted[b]	(3) Adjusted[c]	(4) Adjusted[d]
Herpes Type				
One types vs No herpes (HSV-1)	1.35[0.81,2.24]	1.35[0.81,2.24]	0.67[0.35,1.30]	0.75[0.38,1.47]
Two types vs No herpes (HSV-1 + HSV-2)	2.65[1.48,4.73]***	2.65[1.48,4.73]***	0.79[0.35,1.77]	0.87[0.36,2.13]
Age Group (35~49 vs 20~34)	4.81[3.04,7.63]***	1.12[0.66,1.90]	5.51[2.61,11.65]***	4.68[2.25,9.71]***
		1.84[0.99,3.39]*		
Gender (Male vs Female)	0.89[0.60,1.31]	1.35[0.82,2.23]	-	-
		2.66[1.53,4.60]***		
Race (nonWhite vs White)	0.84[0.58,1.22]	1.44[0.86,2.42]	-	-
		3.02[1.61,5.68]***		
Education (high school vs college or higher)	1.62[1.08,2.42]**	1.27[0.75,2.15]	-	-
		2.40[1.31,4.41]***		
Household Income		0.93[0.52,1.66]		
		1.50[0.74,3.04]		
<$25,000 vs >$55,000	2.24[1.30,3.86]***		2.53[1.47,4.36]***	2.39[1.37,4.18]***
$25,000~54,999 vs >$55,000	1.73[0.97,3.08]*		1.97[1.10,3.54]**	1.83[0.97,3.46]*
Smoking (Smoker vs Nonsmoker)	3.66[2.42,5.53]***	1.25[0.75,2.09]	2.52[1.42,4.49]***	2.47[1.42,4.31]***
		2.20[1.22,3.96]***		
Blood Pressure (High vs Normal)	2.43[1.41,4.20]***	1.16[0.69,1.97]	-	-
		2.72[1.50,4.95]***		
BMI		1.38[0.81,2.34]		
		2.45[1.30,4.62]***		
Overweight vs Normal	1.97[1.12,3.46]**		-	3.17[1.44,6.95]***
Obesity vs Normal	3.25[1.89,5.62]***		-	3.68[1.72,7.91]***
Blood Cholesterol Level		1.34[0.80,2.22]		
		2.60[1.45,4.64]***		
Borderline high vs Desirable	1.07[0.69,1.66]		-	-
High levels vs Desirable	1.43[0.78,2.60]		-	-

C reactive protein		1.33[0.80,2.21] 2.54[1.42,4.56]***		
High or Average vs Low or Normal	1.89[1.12,3.20]**		-	-
Ferritin		1.24[0.63,2.43] 2.63[1.34,5.18]***		
Normal levels vs Low levels	1.36[0.61,3.04]		-	-
High levels vs Low levels	2.97[1.21,7.34]**		-	-

--: the predictor not included in the final multiple logistic regression model, *$p < 0.10$, ** $p < 0.05$, ***$p < 0.01$

a: odds ratio (95% CI) btw CVD and each risk factor only.

b: odds ratio (95% CI) btw CVD and HSV adjusted by each risk factor.

c: odds ratio (95% CI) btw CVD and HSV, demographic variables, and smoke in one full model.

d: odds ratio (95% CI) btw CVD and HSV, demographic variables, smoke and all clinical variables in one full model.

Summary

The multivariate regression analyses accounting for sampling design effects and sampling weight indicated that both T2DM and CVD were significantly associated with HSV-2, but not associated with HSV-1, CMV or burden (multiple infections). Results indicated that T2DM and CVD were associated with demographic variables age, household income, blood pressure, BMI, CRP and ferritin; however, only T2DM was associated with gender and race while CVD was associated with education and smoking. Surprisingly, neither T2DM nor CVD was associated with blood cholesterol. Further examination revealed that nonwhite men, aged 35-49, with household income ≤$55,000, with high blood pressure, overweight or obese, with average to high level of CRP, or high ferritin levels were more likely to increase the risk for developing T2DM. Similarly, older individuals aged 35-49, smoking individuals, or those individuals with a high school education background, a household income ≤$25,000 or between $25,000 and ≤$55,000, high blood pressure, overweight or obese, with an average or high CRP level, or high ferritin levels were more likely to increase the risk for developing CVD.

Further examination revealed that gender, age, household income, BMI and ferritin were confounders of the association between T2DM and HSV-2 while age, household income and smoking were cofounders of the association between CVD and HSV-2. Based on the stepwise regression model, the final regression model for describing the relationship between T2DM and HSV-2 was determined after adjusting for the demographic and clinical variables, age, gender, household income, BMI, CRP and ferritin. No association between T2DM and HSV-2 was observed after factoring for all confounding variables. Similarly, no significant association was observed between CVD and HSV-2 after adjusting for age, household income, and BMI. A similar trend was observed between T2DM and CVD and seropositivity to both, HSV-1 and HSV-2. After controlling for all confounding factors, no significant association was observed between HSV-2 and T2DM or CVD and having both HSV-1and HSV-2. However, a significant association was observed between T2DM and CVD and high CRP and ferritin levels across all analyses. Discussion on these findings and their implication on social changed are further discussed in Chapter 5.

Chapter 5

Discussion, Conclusion, and Implications for Social Change

Introduction

The global prevalence and incidence of HSV is of great public health significance (CDC, 2010; Smith & Robinson, 2002; Wald & Corey, 2007; Xu et al., 2006). The ability of HSV to readily adapt to its host (human or animal), reactivate during the lifetime of the host, and induce recurrent autoimmune and inflammatory responses is believed to increase an individual's susceptibility to disease (Kampoli et al., 2011; Lloyd-Jones et al., 2010; Lutsey et al., 2009; Pickup, 2004; van der Kleij et al., 2003). Despite the body of evidence implicating herpes and other viral and bacterial infections in the pathogenesis of T2DM and CVD, most studies have generated inconsistent results (Lutsey et al., 2009; Rupprecht et al., 2001; Sun et al., 2004) and this study is no exception. Outcomes from this study indicated no association and therefore do not support the hypothesis that HSV or multiple infections with HSV and CMV increase the risk of developing either T2DM or CVD. In contrast, findings from this study support the premise that pathogenic inflammation may be a secondary phenomenon and not directly associated with the disease (ASM, 2014).

Discussion

This study was based on the premise that infectious agents can increase chronic inflammation—a risk factor for several common chronic diseases, including T2DM and CVD. The relationship between human pathogenic HSVs and T2DM or CVD was examined to determine whether an association exists or if it is merely a secondary effect.

Based on data availability, the effect of two inflammatory markers, ferritin and CRP, on T2DM and CVD was studied. Established risk factors for both, T2DM and CVD, including hypertension, overweight or obese, and smoking status were also examined in the present study. Data collected by NHANES from 1999-2010 on individuals between the ages 20-49 was used to conduct this study.

According to national trends, the risk of developing T2DM increases with age; is greater among Hispanics, African Americans, Native Americans and Asians; is greater among individuals who are obese or overweight, and have hypertension. (ADA, 2014; CDC, 2014; Geiss et al., 2014; Menke, Rust, Fradkin, Cheng, & Cowie, 2014; Paeratakul, Lovejoy, Ryan, & Bray, 2002). The prevalence of T2DM in this sample was consistent with the overall national trends by gender, age, race/ethnicity, hypertension, and body mass index. A significantly higher prevalence of T2DM was observed among males than females, which supports the new trend in gender distribution of T2DM due to differences in body fat, insulin resistance, sex hormones, and blood glucose levels (Faerch, Borch-Johnsen, Vaag, Jorgensen, & Witte, 2010; Geer & Shen, 2009; Logue et al., 2011; Perreault et al., 2008; Sicree et al., 2008). Further, this study found no relation between smoking and T2DM, which is surprising given the fact that smoking is a well-established risk factor for T2DM. Nonetheless, a possible explanation for this finding is the decline of cigarette smoking among adults in the last decade, particularly among individual with diabetes (Fan et al., 2013).

T2DM is known to increase the risk of CVD and similar risk factors have been associated with both diseases (Grundy, Pasternak, Greenland, Smith, & Fuster, 1999;

Laakso, 2008; Luscher et al., 2003). Consequently, similar results in the prevalence of CVD were expected. The prevalence of CVD in this sample was also consistent with U.S. trends by age, high blood pressure, smoking, and BMI, but not by gender or ethnicity (Hahn, Health, & Chang, 1998). Surprisingly, neither T2DM nor CVD was related to high cholesterol. It is possible that this study may have been limited to detect significant prevalence differences in cholesterol levels among study participants due to sample size, in addition to the multidimensional factors associated with T2DM and CVD (Dokken, 2008; Kalofoutis et al, 2007).

The prevalence of high CRP and ferritin levels in this study was also consistent with national estimates (Khera et al, 2005; Woloshin, & Schwartz, 2005). High CRP levels were observed among females, nonHispanic Blacks and Hispanics. Significantly higher CRP levels were, however, observed among nonHispanic Whites. It is possible that the analysis performed to assess race/ethnicity in this study may have been biased given that the majority of the study participants were nonHispanic Whites. Additionally, race and/or ethnicity in NHANES data is restricted to only a few classifications, including nonHispanic White, nonHispanic Black, Hispanics, and other racial groups, which limit the subgroup analyses in this study. Further, this study also found, although slightly, higher CRP levels among individuals aged 20-34 than in the older group. These unexpected and divergent results may be an indication of a new trend in the distribution of CRP in the U.S. since CVD has been risen among young people due to childhood obesity and diabetes (Freedman, Zuguo, Srinivasan, Berenson, & Dietz, 2007). Based on Freedman and associates (2007) findings, approximately 70% of obese youth between the

ages of 5-17 had at least one risk factor for CVD, including prediabetes and high blood pressure.

Contrary to CRP, the prevalence of ferritin levels in this sample population was predominantly higher among males, nonHispanic Whites, and study participants aged 35-49. Except for the factor race which limitations were previously discussed, these findings align with the overall distribution of serum ferritin reported by WHO (2014). Further analyses indicated that the prevalence of T2DM and CVD (age-adjusted) was significantly higher among individuals with the average to high CRP and high ferritin levels. These results support the use of CRP and ferritin levels as markers of chronic inflammatory disease and potential independent predictors of T2DM and CVD (Foroubi et al., 2007; Halcox et al., 2014; Watson et al., 2012). Whereas gender differences were observed with serum ferritin in this study, these results provide value to the use of serum ferritin in assessing the risk for T2DM and CVD.

A decrease in the prevalence of HSV-1, HSV-2, and CMV over the last decade was observed in this study population, which also shows consistency with the national estimates (CDC, 2010a, 2010b; Smith et al., 2002; Staras et al., 2006; Xu et al., 2006). A similar tendency was observed among participants who had two or three herpes virus infection indicating a possible additive effect, particularly between HSV-1 and HSV-2, since the incidence of genital herpes with HSV-2 has been decreasing while potentially increasing with HSV-1 (Malkin, 2004). All three herpes virus infections were higher among females and individuals aged 35-49; however, the prevalence rates for HSV-2 were twice as high. Significant racial differences were observed in the prevalence of

HSV-2 among nonHispanic Blacks whereas higher prevalence rates of HSV-1 were observed among Hispanics, followed by other racial populations and nonHispanic Blacks as documented in prior studies (Fanfair et al., 2013; Halford et al., 2004; Kriebs, 2008).

Overall, results from unadjusted and adjusted analyses determined an association between T2DM and HSV-2 OR = 1.66 [1.23, 2.23], T2DM and both HSV-1 and HSV-2 OR = 1.99 [1.25, 3.18], CVD and HSV-2 OR = 2.19 [1.47, 3.27] and CVD and both HSV-1 and HSV-2 OR = 2.65 65[1.48, 4.73]. Nevertheless, after controlling for all potential confounding variables in one full model, including smoking status, blood pressure, BMI, CRP, and serum ferritin, the associations were no longer significant. There was also no association between T2DM or CVD and HSV-1, CMV and burden of infection ≥1. These results support preceding evidence that no association exists between HSV and CMV and T2DM or CVD. However, all the covariates included in the final regression analyses showed a significant association with T2DM and CVD. These findings are consistent with the existing evidence that established risk factors such as hypertension, overweight or obese, and smoking status are associated with T2DM and CVD.

Similar differing results have been reported in preceding studies investigating the association of multiple pathogens with chronic diseases. Lutsey and colleagues (2009) reported a high prevalence of diabetes among individuals with CMV OR = 1.73 [1.06, 2.84], HSV-1 and HSV-2 OR = 2.73 [1.30, 5.75] and with more than 3 pathogens OR = 1.68 [1.07, 2.64]; however, these associations were no longer significant after adjusting for selected demographic variables CMV OR = 1.02 [0.60, 1.72], HSV-1 and HSV-2

OR = 1.50 [0.68, 3.28], and ≥ 3 pathogens OR = 0.81 [0.48, 1.37]. Null results were also documented by Mendy and colleagues (2013) who conducted a study with the same NHANES data (1999-2010) and age group 20-49 to investigate the relationship between CVD and HSVs. No association was found between CVD and HSV-1 OR = 1.13 [95% CI = 0.79-1.62]. Whereas a positive association between HSV-2 and premature CVD OR = 1.56 [95% CI = 1.09-2.21] was reported in this study, this association could be debated as a weak association and may need further examination (Rosenthal, 1996). Other preceding work investigating the link between pathogens, socioeconomic position (SEP) and CVD reported no association between CVD and HSV-1, CVD and both HSV-1 and HSV-2, or CVD and CMV after adjusting for SEP (Simanek, Dowd, & Aiello, 2009). In contrast, CMV was identified as a possible mediator between socioeconomic and CVD and a co-factor in atherosclerosis (Simanek et al, 2009).

Despite the null findings, the present study provides additional support to the existing literature that there is no association between T2DM or CVD and the studied herpes virus infections. It is possible, however, that these infectious agents are possible mediators in the inflammation process and co-factors in the early stage of the disease as reported in previous work (Bassols et al., 2009; Vlassara et al, 2002; Watts et al., 2008). Further, the null results of this study may be related to the study and analysis design. Although this study used a large sample size, subanalyses lowered the numbers and may have biased results. Further, a stepwise regression analysis was utilized since it allows for predictive variables to be added sequentially and altogether in a full model. A few controversial points, however, have been made with regards to stepwise regression,

including tests bias, type I error, distribution of F-statistic and over-simplifications of final models (Copas, 1983; Hurvich, & Tsai, 1990; Roecker, 1991; Wilkinson, & Dallal, 1981). Nevertheless, all predictive variables in this study were manually selected; therefore, the issues mentioned above should not have influenced the results. Other potential factors that could have affected the results are variable manipulations in the analyses. The variable race/ethnicity, for instance, was recategorized from a multiple levels variable to a binary variable due to analysis constrains. Yet, given that most of this study population were nonHispanic Whites, aggregating all the underestimated populations should have in fact canceled out any effects. Additionally, this study looked at the distribution of all three herpes viruses (HSV-1, HSV-2 and CMV) by selected demographic variables, but not across all studied independent variables and covariates. It may be possible that the results may have been affected by some variable distribution; yet, the focus of the study was on two dependent variables, T2DM and CVD, and main covariates indicated normal distribution. Furthermore, the cutoff points for measuring certain biochemical parameters used in this study may have been different from those used in previous studies, which may have caused the differing results.

Nevertheless, based on the evidence of this study, which associations were contributed to confounding, it is presumed that potential confounding factors for T2DM and CVD, other than demographic factors, were not adequately controlled in some of the previous studies. It is also assumed that other factors such as differences in settings, location, and study design may be related to the discrepancy between this study's results and those preceding. Some of the limitations documented in prior works investigating the

role of infection in the prevalence of T2DM and CVD includes clinical populations, age/race-specific, and small sample sizes, which may preclude general population inferences (Lutsey et al., 2009; Paeratakul et al., 2002; Roberts et al., 2005a, 2005b; Sun et al., 2004). When compared to other cross-sectional studies, the present study does align for the most part with reported results (Lutsey et al., 2009; Mendy et al, 2013). Whereas causal factor cannot be assumed with the study design, and results were null, this study raises important questions to be addressed in future research.

Limitations

This study used multiple years of NHANES data which requires different weights for analysis and is particularly important for variance estimation. This, however, resulted in a significant increase in the number of missing and a decrease in sample size across most of the studied variables. Further, the fact that CRP and ferritin levels are potentially linked to both diseases made it difficult to determine their true utility for each disease in this study. Additionally, data for these and other inflammatory markers were not consistently collected by NHANES throughout the 12 years of data collection period, making it difficult to make unbiased comparison analysis and inferences.

Implications for Social Change

Despite the null results, this study provides the medical community and the public health field with valuable information that can be translated into clinical practice. Although there is no definitive evidence that lowering CRP or serum ferritin levels can reduce the risk for diabetes or CVD, it has been documented that managing and controlling traditional risk factors, such as blood pressure, nutrition, exercise, and

smoking, often lead to lowering these inflammatory markers (Ridker, 2000; Ziegler, 2005). Existing guidelines could be expanded for primary and secondary prevention of CVD and T2DM using CRP as a prognostic tool for CVD (Ballantyne et al., 2005; Blake et al., 2002; Davison et al., 2011; Khera et al., 2005; Ridker, 2000) and serum ferritin as a marker for T2DM and CVD even in healthy individuals (Forouhi et al, 2007; Olesnevich et al., 2012; Ossewaarde, 1998; Ponikowska et al., 2013). Although CPR testing for CVD risk assessment continues to be a subject of debate (Capuzzi & Freeman, 2007), using CRP as a marker for inflammatory disease is a widely accepted means for assessing metabolic syndrome risk and diabetes (Ballantyne et al., 2005; Blake et al., 2002; Ridker, 2000). CPR has also been used to investigate the role of pathogen burden in relation to increased risk for atherosclerosis (Zhu et al., 1999).

Implications for Future Research

Findings from this study expand on the existing evidence that no association exists between herpes viruses (HSV-1, HSV-2, and CMV) and T2DM and CVD. The high prevalence of HSV-2 observed among individuals with T2DM and CVD, however, does raise the question whether infections agents have an etiologic role in disease development or if having a chronic disease increases the susceptibility to infection. Conflicting results underscore the need for more collaborative research across diseases like T2DM, CVD and HSV which are traditionally housed in separate silos for research and funding. Although no association was found in this study; certainly, conflicting growing evidence investigating the relation between infectious agents, particularly lifelong viruses, and chronic inflammatory diseases calls for causality research.

Longitudinal studies are particularly useful in detecting changes in a population over time, and the best method for thoroughly explore whether an association really exists between pathogens and chronic diseases or if it is merely an epiphenomenon. Understanding this relationship is imperative for developing preventive measures and treatment for T2DM and CVD. Further, the effect of the studied inflammatory markers (CRP and serum ferritin) as potential predictors for T2DM and CVD and mediators in the activation of inflammation still is unclear (Laakso, 2008; Vlassara et al., 2002; Watson et al., 2012; Watts et al., 2008). Further investigation is needed to advance understanding of the interrelationship between pathogenic agents, inflammation and chronic diseases. Last, high-risk groups often predisposed to chronic disease-related complications and susceptible to infections should be closed examined in future studies.

Conclusion

This study is the first to investigate the association between HSV and two multifactorial chronic diseases for which the primary etiology is unknown, T2DM and CVD. The present work also examined the relationship between two inflammatory markers (CRP and serum ferritin) and T2DM and CVD. Twelve years of cross-sectional data were used to generate a representative and acceptable sample size for this study. Results from this national representative sample indicated that being infected with HSVs, CMV, or with two or three herpes viruses does not increase the risk for developing T2DM or CVD. These findings suggest that infection may be a secondary phenomenon and is not directly associated with disease. However, considering the well-established connection between infection-induced autoimmune inflammatory response and disease, it

is presumed that perhaps pathogens may function as mediators or co-factors in the inflammatory response process, but not directly involved in the etiology of the disease. While causal studies may provide more precise information and better opportunities to assess, diagnose, and treat T2DM and CVD, the complexity of these two top leading causes of death in U.S. leads to the question if developing and implementing more effective prevention measures would be the best approach to address these two common chronic diseases.

Despite this study's marginal findings on infection and chronic disease, additional results from this study showed an association between inflammatory markers and T2DM and CVD. These results suggest that individuals with high levels of CRP or serum ferritin may be at higher risk of developing T2DM or CVD; these findings are of great significance mainly because the potential role that inflammatory markers may play in chronic inflammation and disease. Inflammatory markers and other biomarkers have been used for generations by scientists as disease status indicators; however, their reliability and validity as predictive tools for inflammatory diseases are still in debate. Whereas sensitivity and specificity measurements of biomarkers may be needed for determining progression and severity of a disease, the application of biomarkers should be included in routine screening tests to assist in disease prediction, diagnosis, management, and treatment.

References

Abramowitz, L., Jacquard, A.C., Jaroud, F., Haesebaert, J., Siproudhis, L., Pradat, P…Denis, F. (2010). Human papillomavirus genotype distribution in anal cancer in France: The EDiTH V study. *International Journal of Cancer, 129*(2), 433-439. Doi: 10.1002/ijc.25671

Aiello, A. E., Diez-Roux, A., Noone, A. M., Ranjit, N., Cushman, M., Tsai, M. Y., & Szklo, M. (2009). Socioeconomic and psychosocial gradients in cardiovascular pathogen burden and immune response: the multi-ethnic study of atherosclerosis. *Brain, Behavior, and Immunity*, *23*(5), 663-671. doi: 10.1016/j.bbi.2008.12.006

Altman, R. (2003). Risk factors in coronary atherosclerosis athero-inflammation: the meeting point. *Thrombosis Journal, 1*(4), 1-17.

American Diabetes Association. (2003). Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care*, *26,* 5-20. doi:10.2337/diacare.26.2007.S5

American Diabetes Association. (2014). Data from the National Diabetes Statistics Report. Retrieved 11/10/2014 from http://www.diabetes.org/diabetes-basics/statistics/

American Society for Microbiology. (2014). Potential role of infections in chronic inflammatory diseases. Retrieved 12/13/2014 from http://forms.asm.org/microbe/index.asp?bid=38605

Bakker, G. C., van Erk, M. J., Pellis, L., Wopereis, S., Rubingh, C. M., Cnubben, N., Kooistra, T., Van Ommen, B., & Hendriks, H. (2010). An antiinflammatory dietary mix modulates inflammation and oxidative and metabolic stress in overweight men: a nutrigenomics approach. *American Journal of Clinical Nutrition, 91*(4), 1044-1059. doi: 10.3945/ajcn.2009.28822

Ballantyne, C. M., & Nambi, V. (2005). Markers of inflammation and their clinical significance. *Atherosclerosis Supplements, 6*(2), 21-29.

Banerjee, K., & Rouse, B. T. (2007). Immunopathological aspects of HSV infection. In A. Arvin, G. Campadelli-Fiume, E. Mocarski , P.S. Moore, B. Roizman, R. Whitley, & K. Yamanishi. (Eds.), *Human Herpesviruses: Biology, therapy, and immunoprophylaxis*. Cambridge, England: Cambridge University Press.

Barker, D. J. P. (1995). Fetal Origins of Coronary Heart Disease. *British Medical Journal, 311*(6998), 171-174.

Barzilai, O., Sherer, Y., Ram, M., Izhaky, D., Anaya, J. M., & Shoenfeld, Y. (2007). Epstein-Barr virus and cytomegalovirus in autoimmune diseases: are they truly notorious? A preliminary report. *Annals of the New York Academy of Science, 1108*, 567-577. doi: 10.1196/annals.1422.059

Barzilay, J. I., Abraham, L., Heckbert, S. R., Cushman, M., Kuller, L. H., Resnick, H. E., & Tracy, R.P. (2001). The relation of markers of inflammation to the development of glucose disorders in the elderly: the Cardiovascular Health Study. *Diabetes, 50*(10), 2384-2389. doi: 10.2337/diabetes.50.10.2384

Bassols, J., Moreno, J. M., Ortega, F., Ricart, W., & Fernandez-Real, J. M. (2009). Characterization of herpes virus entry mediator as a factor linked to obesity [Electronic Version]. *Obesity: A Research Journal*, *18*, 239-246. doi:10.1038/oby.2009.250

Bauer, B., & Meyer, T. (2011). The Human Gastric Pathogen Helicobacter pylori and Its Association with Gastric Cancer and Ulcer Disease [Electronic Version]. *Ulcers*, *2011*, 23. doi: 10.1155/2011/340157

Belshe, R. B., Leone, P. A., Bernstein, D. I., Wald, A., Levin, M. J., & Stapleton, J. T. (2012). Efficacy results of a trial of a herpes simplex vaccine. *The New England Journal of Medicine*, *366*(1), 34–43. doi: 10.1056/NEJMoa1103151

Benditt, E. P., Barrett, T., & McDougall, J. K. (1983). Viruses in the etiology of atherosclerosis. *Proceedings of the National Academy of Scences in the United States of America, 80*(20), 6386-6389.

Binkley, P.F., Cooke, G.E., Lesinski, A., Taylor, M., Chen, M., Laskowski, B…Glaser, A. (2013). Evidence for the role of Epstein Barr virus infections in the pathogenesis of acute coronary events. *PLoS ONE, 8* (1), e54008. doi: 10.1371/journal.pone.0054008

Blake, G. J., & Ridker, P. M. (2002). Inflammatory bio-markers and cardiovascular risk prediction. *Journal of Internal Medicine, 252*(4), 283-294. doi: 10.1046/j.1365-2796.2002.01019.x

Bonow RO, M., Mann, D., Zipes, D., & Libby, P. (2011). Primary and secondary prevention of coronary heart disease. In R. P. Gaziano, J.M., Libby, P. (Ed.),

Braunwald's Heart Disease: A Textbook of Cardiovascular Medicine. Philadelphia, PA: Saunders Elsevier.

Bosch, F. X., Lorincz, A., Munoz, N., Meijer, C. J., & Shah, K. V. (2002). The causal relation between human papillomavirus and cervical cancer. *Journal of Clinical Pathology, 55*(4), 244-265.

Bradley, H., Markowitz, L.E., Gibson, T., & McQuillan, G.M. (2014). Seroprevalence of herpes simplex virus types 1 and 2-United States, 1999-2010. *Journal of Infectious Diseases, 209*(3), 325-33. doi: 10.1093/infdis/jit458

Braly, J., & Holford, P. (2003). *The H Factor Solution: Homocysteine, the Best Single Indicator of Whether You Are Likely to Live Long or Die Young* (1st ed.). North Bergen, NJ: Basic Health Publications.

Britt, W. J., & Mach, M. (1996). Human cytomegalovirus glycoproteins. *Intervirology, 39*(5-6), 401-412.

Burnett-Hartman, A. N., Newcomb, P. A., & Potter, J. D. (2008). Infectious agents and colorectal cancer: a review of Helicobacter pylori, Streptococcus bovis, JC virus, and human papillomavirus. *Cancer Epidemiology, Biomarkers, and Prevention, 17*(11), 2970-2979. doi:10.1158/1055-9965.Epi-08-0571

Campbell, L. A., & Kuo, C. C. (2004). Chlamydia pneumoniae--an infectious risk factor for atherosclerosis? *Nature Reviews Microbiology, 2*(1), 23-32. doi: 10.1038/nrmicro796

Capuzzi, D.M., & Freeman, J.S. (2007). C-reactive protein and cardiovascular risk in the metabolic syndrome and type2 diabetes: controversy and challenge. Clinical Diabetes, 25(1), 16-22. doi: 10.2337/diaclin.25.1.16

Centers for Disease Control and Prevention. (2010a). CDC Study Finds U.S. Herpes Rates Remain High [Press release]. Retrieved 10/14/2012 from http://www.cdc.gov/nchhstp/newsroom/HSV-2pressrelease.html

Centers for Disease Control and Prevention. (2010b). Seroprevalence of Herpes Simplex Virus Type 2 Among Persons Aged 14--49 Years --- United States, 2005--2008. *MMWR Morb Mortal Wkly Rep, 59*(15), 456-459.

Centers for Disease Control and Prevention. (2012). National Centers for Health Statistics. Retrieved 05/15/2012 from http://www.cdc.gov/nchs/

Centers for Disease Control and Prevention. (2014). Rate per 100 of Civilian, Noninstitutionalized Population with Diagnosed Diabetes, by Age, United States, 1980–2011. Retrieved 9/12/2014 from http://www.cdc.gov/diabetes/statistics/prev/national/figbyage.htm

Chayavichitsilp, P., Buckwalter, J. V., Krakowski, A. C., & Friedlander, S. F. (2009). Herpes simplex. *Pediatrics in Review / American Academy of Pediatrics, 30*(4), 119-129.doi:10.1542/pir.30-4-119

Chen, S., de Craen, A., Raz, Y., Derhovanessian, E., Vossen, A., Westerndorp, R. Pawelec, G., Maier, A. (2012). Cytomegalovirus seropositivity is associated with glucose regulation in the oldest old. Results from the Leiden 85-plus Study. *Immunity & Ageing, 9*(1), 18. doi: 10.1186/1742-4933-9-18

Chentoufi, A.A., Dervillez, X., Rubbo, P.A., Kuo, T., Zhang, X., Nagot, N., Tuaillon, E. Van De Perre, P., Nesburn, A.B., & BenMohamed, L. (2012). Current trends in negative immune-synergy between two sexually transmitted infections viruses: HIV-1 and HSV-1/2. *Current Trends in Immunology, 13*, 51-68.

Christen, S., Hagen, T. M., Shigenaga, M. K., & Ames, B. N. (1999). Chronic inflammation, mutation, and cancer. In J. Parsonet (Ed.), *Microbes and malignancy: infection as a cause of human cancers* (pp. 35-88). New York: Oxford University Press.

Cone, J. B. (2001). Inflammation. *American Journal of Surgery, 182*(6), 558-562.

Conrady, C. D., Drevets, D. A., & Carr, D. J. (2011). Herpes simplex type I (HSV-1) infection of the nervous system: is an immune response a good thing? *Journal of Neuroimmunology, 220*(1-2), 1-9. doi: 10.1016/j.neuroim.2009.09.013

Copas, J.B. (1983). Regression, prediction and shrinkage. *Royal Statistical Society, Series B, 45,* 311–354.

Crook, M. A., Tutt, P., Simpson, H., & Pickup, J. C. (1993). Serum sialic acid and acute phase proteins in type 1 and type 2 diabetes mellitus. *Clinica Chimica Acta, 219*(1-2), 131-138.

Danesh, J., Collins, R., & Peto, R. (1997). Chronic infections and coronary heart disease: is there a link? *Lancet, 350*(9075), 430-436.

Davidson, M.H., Jacobson, T.A., Bittner, V.A., Braun, L.T., Brown, A.S., Brown, W.V…Dicklin, M.R. (2011). Clinical utility of inflammatory markers and

advanced lipoprotein testing: Advice from an expert panel of lipid specialists. *Journal of Clinical Lipidology* (5), 338-367.

de Martel, C., & Franceschi, S. (2009). Infections and cancer: Established associations and new hypotheses. *Critical Reviews in Oncology/Hematology*, *70*(3), 183-194.: doi: 10.1016/j.critevonc.2008.07.021

De Tiege, X., Rozenberg, F., & Heron, B. n. d. (2008). The spectrum of herpes simplex encephalitis in children. *European Journal of Paediatric Neurology: EJPN: Official Journal Of The European Paediatric Neurology Society, 12*(2), 72-81. doi: 10.1016/j.ejpn.2007.07.007

Dokken, B.B. (2008). The pathophysiology of cardiovascular disease and diabetes: beyond blood pressure and lipids. *Diabetes Spectrum, 21*(3), 160-165. doi:10.2337/diaspect.21.3.160

Draper, N., & Smith, H. (1981). *Applied Regression Analysis,* (2nd ed), New York, NY: John Wiley & Sons, Inc.

Duncan, B.B., Schmidt, M.I., Chambless, L.E., Folsom, A.R., Carpenter, M., & Heiss, G. (2000). Fibrinogen, other putative markers of inflammation, and weight gain in middle-aged adults—the ARIC study: Atherosclerosis Risk in Communities. *Obesity Research and Clinical Practice.* 8:279–286, 2000

Dziurzynski, K., Wei, J., Qiao, W., Hatiboglu, M. A., Kong, L. Y., Wu, A. Heimberger, A.B. (2012). Glioma-associated cytomegalovirus mediates subversion of the monocyte lineage to a tumor propagating phenotype. *Clinical Cancer Research, 17*(14), 4642-4649. doi: 10.1158/1078-0432.CCR-11-0414

Epstein, S. E., Zhou, Y. F., & Zhu, J. (1999). Infection and atherosclerosis: emerging mechanistic paradigms. *Circulation, 100*(4), 20-28. doi:10.1161/01.CIR.100.4.e20

Epstein, S. E. (2002). The multiple mechanisms by which infection may contribute to atherosclerosis development and course. *Circulation Research, 90*(1), 2-4.

Erdmann, E. (2005). Diabetes and cardiovascular risk markers. *Current Medical Research and Opinion, 21 Suppl 1*, S21-28.

Espinola-Klein, C., Rupprecht, H. J., Blankenberg, S., Bickel, C., Kopp, H., Victor, A., Hafner, G., Prellwitz, W., Schlumberger, W., & Meyer, J. (2002). Impact of infectious burden on progression of carotid atherosclerosis. *Stroke, 33*(11), 2581-2586.

Færch K.,Borch-Johnsen, K,, Vaag, A,, Jørgensen, T., & Witte, D. (2010) Sex differences in glucose levels: a consequence of physiology or methodological convenience? The Inter99 study. *Diabetologia*, 53: 858-865.

Fan, A.Z., Rock, V., Zhang, X., Li, Y., Elam-Evans, L., & Balluz, L. (2013). Trends in cigarette smoking rates and quit attempts among adults with and without diagnosed diabetes, United States, 2001–2010. *Preventing Chronic Disease, 10* (120259).

Fanfair, R.N., Zaidi, A., Taylor, L.D., Xu, F., Gottlieb, S., Markowitz, L. (2013). Trends in seroprevalence of herpes simplex virus type 2 among nonHispanic blacks and nonHispanic whites aged 14 to 49 years--United States, 1988 to 2010, 40 (11), 860-864. doi: 10.1097/OLQ.0000000000000043

Fatahzadeh, M., & Schwartz, R. A. (2007). Human herpes simplex virus infections: epidemiology, pathogenesis, symptomatology, diagnosis, and management. *Journal of the American Academy of Dermatology, 57*(5), 737-763.

Fearon, D. T. (1997). Seeking wisdom in innate immunity. *Nature, 388*(6640), 323-324.

Fernandez-Real, J. M., & Ricart, W. (2003). Insulin resistance and chronic cardiovascular inflammatory syndrome. *Endocrine Reviews, 24*(3), 278-301. doi: 10.1210/er.2002-0010

Fernandez-Real, J.M., Ferri, M.-J., Vendrell, J., & Ricart, W. (2007). Burden of infection and fat mass in healthy middle-aged men. *Obesity,* (Silver Spring, Md.), *15*(1), 245-252.

Festa, A., D'Agostino, R., Jr., Tracy, R. P., & Haffner, S. M. (2002). Elevated levels of acute-phase proteins and plasminogen activator inhibitor-1 predict the development of type 2 diabetes: the insulin resistance atherosclerosis study. *Diabetes, 51*(4), 1131-1137. doi: 10.2337/diabetes.51.4.1131

Firestein, G. S. (2011). Mechanisms of inflammation and tissue repair. In L. Goldman & A. I. Schafer (Eds.), Goldman's *Cecil Medicine* (24th ed.), Philadelphia, PA: Saunders Elsevier.

Fong, I. W. (2000). Emerging relations between infectious diseases and coronary artery disease and atherosclerosis. *Canadian Medical Association Journal, 163*(1), 49-56.

Forouhi, N.G., Harding, A.H., Alison, M., Sandhu, M.S., Welch, A., Luben, R…& Wareham, N.J. (2007). Elevated serum ferritin levels predict new-onset type 2

diabetes:results from the EPIC-Norfolk prospective study. *Diabetologia, 50*, 949-956. doi:10.1007/s00125-007-0604-5

Fowler, K. B., Stagno, S., Pass, R. F., Britt, W. J., Boll, T. J., & Alford, C. A. (1992). The outcome of congenital cytomegalovirus infection in relation to maternal antibody status. *The New England Journal of Medicine, 326*(10), 663-667. doi:10.1056/NEJM199203053261003

Freeman, D. J., Norrie, J., Caslake, M. J., Gaw, A., Ford, I., Lowe, G. D., Sattar, N. (2002). C-reactive protein is an independent predictor of risk for the development of diabetes in the West of Scotland Coronary Prevention Study. *Diabetes, 51*(5), 1596-1600.

Freedman, D.S., Zuguo, M., Srinivasan, S.R., Berenson, G.S., & Dietz, W.H. (2007). Cardiovascular risk factors and excess adiposity among overweight children and adolescents: the Bogalusa Heart Study. *Journal of Pediatrics, 150(*1):12–17.

Freeman, E.E., Weiss, H. A., Glynn, J. R., Cross, P. L., Whitworth, J. A., & Hayes, R. J. (2006). Herpes simplex virus 2 infection increases HIV acquisition in men and women: systematic review and meta-analysis of longitudinal studies. *Aids, 20*(1), 73-83.

Friedman, H.M., Macarak, E.J., MacGregor, R.R., Wolfe, J., & Kefalides N. A. (1981). Virus infection of endothelial cells. *Journal of Infectious Diseases***,** *143*(2), 266–273.

Gabay, C., & Kushner, I. (1999). Acute-phase proteins and other systemic responses to inflammation. *The New England of Journal Medicine, 340*(6), 448-454.

Gabrylewicz, B., Mazurek, U., Ochala, A., Sliupkas-Dyrda, E., Garbocz, P., Pyrlik, A…Tendera, M. (2003). Cytomegalovirus infection in acute myocardial infarction. Is there a causative relationship? *Kardiologia Polska*, *59*(10), 283-292.

Galkina, E., KLaus, L. (2009). Immune and Inflammatory Mechanisms of Atherosclerosis. *Annual Review of Immunology*, *27*, 165-197.

Geer, E.B., Shen, W. (2009). Gender differences in insulin resistance, body composition, and energy balance. *Gender Medicine*, 6 Suppl 1: 60-75.

Geiss, L.S. Wang, J., Cheng, Y.J., Thompson, T.J., Barker, L., Li, Y., Albright, A.L., & Gregg, E.W. (2014). Prevalence and incidence trends for diagnosed diabetes among adults aged 20 to 79 years, United States, 1980-2012. *Journal of the American Medical Association, 312* (12), 1218-1226. doi:10.1001/jama.2014.11494

Gern, J. E. (2009). Rhinovirus and the initiation of asthma. *Current Opinion in Allergy and Clinical Immunology*, *9*(1), 73-78. doi:10.1097/ACI.0b013e32831f8f1b

Ghazizadeh, R., Shimizu, H., Tosa, M., & Ghazizadeh, M. (2010). Pathogenic mechanisms shared between psoriasis and cardiovascular disease. *International Journal of Medical Sciences, 7*(5), 284-289.

Gómez, E., Laurés, A., Baltar, J. M., Melón, S., Díez, B., & de Oña, M. (2005). Cytomegalovirus replication and "herpesvirus burden" as risk factor of cardiovascular events in the first year after renal transplantation. *Transplantation Proceedings*, *37*(9), 3760-3763.

Grattan, M. T., Moreno-Cabral, C. E., Starnes, V. A., Oyer, P. E., Stinson, E. B., & Shumway, N. E. (1989). Cytomegalovirus infection is associated with cardiac allograft rejection and atherosclerosis. *Journal of American Medical Association, 261*(24), 3561-3566. doi:10.1001/jama.1989.03420240075030

Grayston, J. T., Kuo, C. C., Campbell, L. A., & Benditt, E. P. (1993). Chlamydia pneumoniae, strain TWAR and atherosclerosis. *European Heart Journal, 14 Suppl K*, 66-71.

Greenland, P., Alpert, J., Beller, G., Benjamin, E., Budoff, M., & Fayad, Z. (2012). 2010 ACCF/AHA guideline for assessment of cardiovascular risk in asymptomatic adults: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines [Electronic version]. *Circulation. 122*, 584-636.

Gregor, M.F., Hotamisligil, G.S. (2011). Inflammatory mechanisms in obesity. *Annual Review of Immunology*. 29:415-45. doi: 10.1146/annurev-immunol-031210-101322

Grundy, S.M., Pasternak, R., Greenland, P., Smith, S., & Fuster, V. (1999). Assessment of cardiovascular risk by use of multiple-risk-factor assessment equations. *Circulation, 100,* 1481-1492. doi:10.1161/01.CIR.100.13.1481

Gruys, E., Toussaint, M., Niewold, T., & Koopmans, S. (2005). Acute phase reaction and acute phase proteins. *Journal of Zhejiang University Science B, 6*(11), 1045-1056.

Gyorkey, F., Melnick, J. L., Guinn, G. A., Gyorkey, P., & DeBakey, M. E. (1984). Herpesviridae in the endothelial and smooth muscle cells of the proximal aorta in arteriosclerotic patients. *Experimental and Molecular Pathology, 40*(3), 328-339.

Hahn, D. L., Dodge, R. W., & Golubjatnikov, R. (1991). Association of Chlamydia pneumoniae (strain TWAR) infection with wheezing, asthmatic bronchitis, and adult-onset asthma. *Journal of the American Medical Association, 266*(2), 225-230. doi:10.1001/jama.1991.03470020051031

Hahn, R.A., Health, G.W., & Chang, M.H. (1998). Cardiovascular disease risk factors and preventive practices among adults—U.S., 1994: a behavioral risk factor atlas. *Morbidity and Mortality Weekly Report, 47*(SS-5), 35-69.

Halcox, J.P., Roy, C., Tubach, F., Banegas, J.R., Dallongeville, J., De Backer, G… & Borghi, C. (2014). C-reactive protein levels in patients at cardiovascular risk: Eurika study. *BMC Cardiovascular Disorders, 25*. doi: 10.1186/1471-2261-14-25

Halford, W. P., Balliet, J. W., & Gebhardt, B. M. (2004). Re-evaluating natural resistance to herpes simplex virus type 1. *Journal of Virology, 78*(18), 10086-10095. doi: 10.1128/JVI.78.18.10086-10095.2004

Heymann, A. D., Chodick, G., Karpati, T., Kamer, L., Kremer, E., Green, M. S…& Shalev, V. (2008). Diabetes as a risk factor for herpes zoster infection: results of a population-based study in Israel. *Infection, 36*(3), 226-230. doi: 10.1007/s15010-007-6347-x

Hocking, R. R. (1976). The Analysis and Selection of Variables in Linear Regression, *Biometrics, 32* (1), 1-49.

Hoffmeister, A., Rothenbacher, D., Bode, G., Persson, K., Marz, W., Nauck, M. A…& Koenig, W. (2001). Current infection with Helicobacter pylori, but not seropositivity to Chlamydia pneumoniae or cytomegalovirus, is associated with an atherogenic, modified lipid profile. *Arteriosclerosis, Thrombosis and Vascular Biology, 21*(3), 427-432. doi: 10.1161/01.ATV.21.3.427

Holmberg, S. D., Stewart, J. A., Gerber, A. R., Byers, R. H., Lee, F. K., O'Malley, P. M., Nahmias, A.J. (1988). Prior herpes simplex virus type 2 infection as a risk factor for HIV infection. *Journal of the American medical Association, 259*(7), 1048-1050. doi:10.1001/jama.1988.03720070048033

Holmes, K. (1999). *Biology of Herpesviruses*. In P. E. Pertel & P. G. Spear (Eds.), Sexually Transmitted Diseases (3rd ed., pp. 295-307). New York, NY: McGraw Hill Book Co.

Huang, W., Xie, P., Xu, M., Li, P., & Zao, G. (2011). The influence of stress factors on the reactivation of latent herpes simplex virus type 1 in infected mice. *Cell Biochemistry Biophysics, 61*(1), 115-122.

Hunt, R. (2010). *Microbiology and Immunology On-line*. Retrieved Jul 25, 2012, from http://pathmicro.med.sc.edu/virol/herpes.htm

Hurvich, C. M. & C. L. Tsai. (1990). The impact of model selection on inference in linear regression. *American Statistician, 44*, 214–217.

Itzhaki, R. F., & Wozniak, M. A. (2008). Herpes simplex virus type 1 in Alzheimer's disease: the enemy within. *Journal of Alzheimer's Disease, 13*(4), 393-405.

Joh, J., Jenson, A. B., Moore, G. D., Rezazedeh, A., Slone, S. P., Ghim, S. J., Kloecker, G.H. (2010). Human papillomavirus (HPV) and Merkel cell polyomavirus (MCPyV) in nonsmall cell lung cancer. *Experimental and Molecular Pathology, 89*(3), 222-226. doi:10.1016/j.yexmp.2010.08.001

Johnson, R.E., Nahmias, A.J., Magder, L.S., Lee, F.K., Brooks, C.A., Snowden, C.B. (1989). A seroepidemiology survey of the prevalence simplex virus type 2 infection in the United States. *New England Journal of Medicine, 321* (1)7-12.

Jun, H. S., & Yoon, J. W. (2003). A new look at viruses in type 1 diabetes. *Diabetes/olism Research and Reviews, 19*(1), 8-31. doi: 10.1002/dmrr.337

Kalofoutis, C., Piperi, C., Kalofoutis, A., Harris, F., Phoenix, D., & Singh, J. (2007). Type II diabetes mellitus and cardiovascular risk factors: current therapeutic approaches. *Experimental and Clinical Cardiology, 12*(1), 117-28.

Kampoli, A.M., Tousoulis, D., Briasoulis, A., Latsios, G., Papageorgiou, N., Stefanadis, C. (2011). Potential pathogenic inflammatory mechanisms of endothelial dysfunction induced by type 2 diabetes mellitus. *Current Pharmaceutical Design, 17*(37), 4147-4158.

Kannel, W.B., Wolf, P.A., Castelli, W.P., D'Agostino, R.B. (1987). Fibrinogen and risk of cardiovascular disease: the Framingham Study. *Journal of the American Medical Association,258*(9),1183–1186. doi10.1001/jama.1987.03400090067035

Karim, S., Mirza, Z., Kamal, M.A., Abuzenadah, A.M., Azhar, E.I., Al-Qahtani, M.H., Sohrab, S.S. (2014). An association of virus infection with type 2 diabetes and

Alzheimer's disease. *CNS and Neurological Disorders Drug Targets, 13*(3),429-439.

Karjala, Z., Neal, D., & Rohrer, J. (2011). Association between HSV-1 seropositivity and obesity: data from the National Health and Nutritional Examination Survey, 2007-2008. *PLoS One, 6*(5), e19092. doi: 10.1371/journal.pone.0019092

Kesson, A. M. (2001). Management of neonatal herpes simplex virus infection. *Paediatric Drugs, 3*(2), 81-90.

Khanna, K. M., Bonneau, R. H., Kinchington, P. R., & Hendricks, R. L. (2003). Herpes simplex virus-specific memory CD8+ T cells are selectively activated and retained in latently infected sensory ganglia. *Immunity, 18*(5), 593-603.

Khera A., McGuire, D.K., Murphy, S.A., Stanek, H.G., Das, S.R., Vongpatanasin, W., Wians, F.H., Grundy, S.M., & de Lemos, J.A. (2005). Race and gender differences in c-reactive protein levels. *Journal of the American College of Cardiology, 46*(3), 464-469. doi:10.1016/j.jacc.2005.04.051

Kimberlin, D.W. (2004). Neonatal herpes simplex infection. *Clinical Microbiology Reviews Journal. 17*(1)1-13.

Kimberlin, D.W., (2014). Infectous disease and antimicrobe. Retrieved 12/05/2013 from http://www.antimicrobe.org/v30.asp

Koelle, D. M., & Corey, L. (2003). Recent progress in herpes simplex virus immunobiology and vaccine research. *Clinical Microbiology Reviews, 16*(1), 96-113. doi: 10.1128/CMR.16.1.96-113.2003

Kountouras, J., Boziki, M., Gavalas, E., Zavos, C., Grigoriadis, N., Deretzi, G., Venizelos, I. (2009). Eradication of helicobacter pylori may be beneficial in the management of Alzheimer's disease. *Journal of Neurology, 256*(5), 758-767.

Kriebs, J.M. (2008). Understanding herpes simplex virus: transmission, diagnosis, and considerations in pregnancy management. *Journal of Midwifery & Women's Health.* 53(3), 202-208.

Kumel, G., Kirchner, H., Zawatzky, R., Engler, H., Schroder, C. H., & Kaerner, H. C. (1982). Experimental Infection of Inbred Mice with Herpes Simplex Virus. V. investigations with a virus strain nonlethal after peripheral Infection. *Journal of General.Virology, 63*, 315-323.

Kusters, J. G., van Vliet, A. H. M., & Kuipers, E. J. (2006). Pathogenesis of *helicobacter pylori* infection. *Clinical Microbiology Reviews*, *19*(3), 449–490. doi:10.1128/CMR.00054-05

Laakso, M. (2008). Cardiovascular disease in type 2 diabetes from population to man to mechanisms: the Kelly West Award Lecture 2008. *Diabetes Care, 33*(2), 442-449. doi: 10.2337/dc09-0749

Lafferty, W.E. (2002). The changing epidemiology of HSV-1 and HSV-2 and implications for serological testing. *Herpes*, *99*(2), 51-5.

Lawson, J. S., Gunzburg, W. H., & Whitaker, N. J. (2006). Viruses and human breast cancer. *Future Microbiology*, *1*(1), 33-51. doi:10.2217/17460913.1.1.33

Lecube, A., Hernandez, C., Genesca, J., Esteban, J. I., Jardi, R., & Simo, R. (2004). High prevalence of glucose abnormalities in patients with hepatitis C virus infection: a

multivariate analysis considering the liver injury. *Diabetes Care, 27*(5), 1171-1175.

Leone, P. (2005). Reducing the risk of transmitting genital herpes: advances in understanding and therapy. *Current Medical Research Opinion, 21*(10), 1577-1582.

Libby, P., Ridker, P. M., & Maseri, A. (2002). Inflammation and atherosclerosis. *Circulation, 105*(9), 1135-1143. doi:10.1161/hc0902.104353

Liesegang, T. J. (2001). Herpes simplex virus epidemiology and ocular importance. *Cornea, 20*(1), 1-13.

Lima, V. P., de Lima, M. A., Andre, A. R., Ferreira, M. V., Barros, M. A., & Rabenhorst, S. H. (2008). H pylori (CagA) and Epstein-Barr virus infection in gastric carcinomas: correlation with p53 mutation and c-Myc, Bcl-2 and Bax expression. *World Journal of Gastroenterology, 14*(6), 884-891. doi: 10.1097/PDM.0b013e31814e5d8f

Linnavuori, K., & Hovi, T. (1987). Herpes simplex virus as an inducer of interferon in human monocyte cultures. *Antiviral Research, 8*(4), 201-208. doi: 10.1016/0166-3542(87)90074-X

Lloyd-Jones, D. M., Hong, Y., Labarthe, D., Mozaffarian, D., Appel, L. J., Van Horn, L…Rosamond, W.D. (2010). Defining and setting national goals for cardiovascular health promotion and disease reduction: the American Heart Association's strategic Impact Goal through 2020 and beyond. *Circulation, 121*(4), 586-613. doi: 10.1161/CIRCULATIONAHA.109.192703

Logue, J., Walker, J., Colhoun, H., Leese, G.P., Lindsay, R.S., McKnight, J.A…&Sattar, N. (2011) Do men develop type 2 diabetes at lower body mass indices than women? *Diabetologia*, 54: 3003-3006.

Looker, K. J., Garnett, G. P., & Schmid, G. P. (2008). An estimate of the global prevalence and incidence of herpes simplex virus type 2 infection [Electronic Version]. *Bulletin of the World Health Organization, 86*, 737-816.

Luscher, T. F., Creager, M. A., Beckman, J. A., & Cosentino, F. (2003). Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: Part II. *Circulation, 108*(13), 1655-1661. doi: 10.1161/01.CIR.0000089189.70578.E2

Lutsey, P. L., Pankow, J. S., Bertoni, A. G., Szklo, M., & Folsom, A. R. (2009). Serological evidence of infections and Type 2 diabetes: the MultiEthnic Study of Atherosclerosis. *Diabetic Medicine: A Journal of The British Diabetic Association, 26*(2), 149-152. doi: 10.1111/j.1464-5491.2008.02632.x

Mahler, R. J., & Adler, M. L. (1999). Clinical review 102: Type 2 diabetes mellitus: update on diagnosis, pathophysiology, and treatment. *Journal Clinical Endocrinology and Metabolism, 84*(4), 1165-1171. doi: 10.1210/jc.84.4.1165

Malkin, J.E. (2004). Epidemiology of genital herpes simplex virus infection in developed countries. *Herpes,* Suppl 1; 2A-23A.

Mark, K. E., Wald, A., Margaret, A. S., Selke, S., Olin, L., Huang, M.L., & Corey, L. (2008). Rapidly cleared episodes of herpes simplex virus reactivation in

immunocompetent adults. *The Journal of Infectious Diseases, 198*(8), 1141–1149. doi: 10.1086/591913

McClelland, R. S., Wang, C. C., Overbaugh, J., Richardson, B. A., Corey, L., Ashley, R. L., Kreiss, J.K. (2002). Association between cervical shedding of herpes simplex virus and HIV-1. *AIDS, 16*(18), 2425-2430.

McKie, E. A., Brown, S. M., MacLean, A. R., & Graham, D. I. (1998). Histopathological responses in the CNS following inoculation with a non-neurovirulent mutant (1716) of herpes simplex virus type 1 (HSV 1), relevance for gene and cancer therapy. *Neuropathology and Applied Neurobiology, 24*(5), 367-372.doi: 10.1046/j.1365-2990.1998.00133.x

Meade, T.W. (1997). Fibrinogen and cardiovascular disease. *Journal of Clinical Pathology*, *50*(1), 13-15.

Mehta, S. H., Brancati, F. L., Strathdee, S. A., Pankow, J. S., Netski, D., Coresh, J., Szklo, M.,& Thomas, D.L. (2003). Hepatitis C virus infection and incident type 2 diabetes. *Hepatology, 38*(1), 50-56. doi: 10.1053/jhep.2003.50291

Mell, H. K. (2008). Management of oral and genital herpes in the emergency department [Electronic Version]. *Emergency Medicine Clinics of North America, 26*,457-473.

Mendy, A., & Vieira, E.R., & Gasana, J. (2013). Seropositivity to herpes simplex virus type 2, but not type 1 is associated with premature cardiovascular diseases: a population-based cross-sectional study. *Atherosclerosis, 231*(1), 18-21. doi: 10.1016/j.atherosclerosis.2013.08.020

Menke, A., Rust, K.F., Fradkin, J., Cheng, Y.J., & Cowie, C.C. (2014). Associations between trends in race/ethnicity, aging, and body mass index with diabetes prevalence in the United States: a series of cross-sectional studies increase in diabetes prevalence over time. *Annual of Internal Medicine 16*(5), 328-335. doi10.7326/M14-0286

Mingo, R. M., Han, J., Newcomb, W. W., & Brown, J. C. (2012). Replication of herpes simplex virus: egress of progeny virus at specialized cell membrane sites. *Journal of Virology*, *86*(13), 7084-7097. doi: 10.1128/JVI.00463-12

Moynihan, J. A., & Ader, R. (1996). Psychoneuroimmunology: animal models of disease. *Psychosomatic Medicine, 58*(6), 546-558.

Muhlestein, J. B., & Anderson, J. L. (2003). Infectious serology and atherosclerosis: how burdensome is the risk? *Circulation, 107*(2), 220-222. doi: 10.1161/01

Muller, S., Martin, S., Koenig, W., Hanifi-Moghaddam, P., Rathmann, W., Haastert, B., & Kolb, H. (2002). Impaired glucose tolerance is associated with increased serum concentrations of interleukin 6 and co-regulated acute-phase proteins but not TNF-alpha or its receptors. *Diabetologia*, *45*(6), 805-812. doi: 10.1007/s00125-002-0829-2

Nabipour, I., Vahdat, K., Jafari, S. M., Pazoki, R., & Sanjdideh, Z. (2006a). The association of metabolic syndrome and chlamydia pneumoniae, helicobacter pylori, cytomegalovirus, and herpes simplex virus type 1: the Persian Gulf healthy heart study. *Cardiovascular Diabetology, 5*, 25. doi:10.1186/1475-2840-5-25

Nakanishi, N., Yoshida, H., Matsuo, Y., Suzuki, K., & Tatara, K. (2002). White blood-cell count and the risk of impaired fasting glucose or type II diabetes in middle-aged Japanese men. *Diabetologia, 45*(1), 42-48.

Navarro, J. F., & Mora, C. (2005). Role of inflammation in diabetic complications. *Nephrology Dialysis Transplantation, 20*(12), 2601-2604. doi: 10.1093/ndt/gfi155

Nicholson A, Hajjar D. (1998). Herpesvirus in atherosclerosis and thrombosis: etiologic agents or ubiquitous bystanders?. *Arterioscl Thromb Vasc Biol*, *18*(3):339–349.

Nielen, M., van Sijl, A., Peters, M., Verheij, R., Schellevis, F., & Nurmohamed, M. (2012). Cardiovascular disease prevalence in patients with inflammatory arthritis, diabetes mellitus and osteoarthritis: a cross-sectional study in primary care. *BMC Musculoskeletal Disorders, 13*(1), 150. doi: 10.1186/1471-2474-13-150

Nieto, F. J. (1998). Infections and atherosclerosis: new clues from an old hypothesis? *American Journal of Epidemiology, 148*(10), 937-948.

O'Connor, S.M., Taylor, C.E., Hughes, J.M. (2006). Emerging infectious determinants of chronic diseases. *Emerging Infectious Diseases* [serial on the Internet]. 2006 Jul [January 2014]. doi:10.3201/eid1207.060037

Oke, A.J., & Oke, A.A. (2014). Herpes simplex (Hsv–1, 2) seropositivity: A study carried out among diabetic and non-diabetic patients in a community in southwest Nigeria. *Journal of Dental and Medical Sciences, 13*(1), 53-55.

Olesnevich, M.E., Kuczmarski, M.F., Mason, M., Fang, C., Zonderman, A.B., & Evans,

M.M.K. (2012). Serum ferritin levels associated with increased risk for developing CHD in a low-income urban population, *Public Health Nutrition, 15*, 1291–1298.

Onorato, I. M., Morens, D. M., Martone, W. J., & Stansfield, S. K. (1985). Epidemiology of cytomegaloviral infections: recommendations for prevention and control. *Reviews of Infectious Diseases, 7*(4), 479-497.

Oshaug, A., Bugge, K. H., Bjonnes, C. H., Borch-Iohnsen, B., & Neslein, I. L. (1995). Associations between serum ferritin and cardiovascular risk factors in healthy young men. A cross sectional study. *European Journal of Clinical Nutrition, 49(6), 430-438.*

Ossewaarde, J. M., Feskens, E. J., De Vries, A., Vallinga, C. E., & Kromhout, D. (1998). Chlamydia pneumoniae is a risk factor for coronary heart disease in symptom-free elderly men, but Helicobacter pylori and cytomegalovirus are not. *Epidemiology and Infection, 120*(1), 93-99.

Paeratakul, S., Lovejoy, J.C., Ryan, D.H., & Bray, G.A. (2002). The relation of gender, race, and socioeconomic status to obesity and obesity comorbidities in a sample of US adults. *International Journal of Obesity, 26*(9), 1205-1210.

Pannacciulli, N., De Pergola, G., Giorgino, F., & Giorgino, R. (2002). A family history of Type 2 diabetes is associated with increased plasma levels of C-reactive protein in non-smoking healthy adult women. *Diabetic Medicine: A Journal Of The British Diabetic Association.* doi: 10.1046/j.1464-5491.2002.00770.x

Pepose, J. S. (1991a). External ocular herpesvirus infections in immunodeficiency. *Current Eye Research, 10 Suppl*, 87-95.

Pepose, J. S. (1991b). Herpes simplex keratitis: role of viral infection versus immune response. *Survey of Ophthalmology*, *35*(5), 345-352. doi: 10.1099/vir.0.81246-0

Perreault, L., Ma, Y., Dagogo-Jack, S, et al., (2008) Sex Differences in Diabetes Risk and the Effect of Intensive Lifestyle Modification in the Diabetes Prevention Program. *Diabetes care*, 31: 1416-1421.

Phillips, D. I., Walker, B. R., Reynolds, R. M., Flanagan, D. E., Wood, P. J., Osmond, C… & Whorwood, C.B. (2000). Low birth weight predicts elevated plasma cortisol concentrations in adults from 3 populations. *Hypertension, 35*(6), 1301-1306. doi: 10.1161/01.HYP.35.6.1301

Pickup, J. C., Mattock, M. B., Crook, M. A., Chusney, G. D., Burt, D., & Fitzgerald, A. P. (1995). Serum sialic acid concentration and coronary heart disease in NIDDM. *Diabetes Care, 18*(8), 1100-1103.

Pickup, J. C., Chana, T., Mattock, M. B., Samuel, A., & Mather, H. M. (1996). Serum sialic acid concentrations in Asian diabetic patients in the UK. *Diabet Med, 13*(3), 284-285. doi: 10.1002/(SICI)1096-9136(199603)13:3<284::AID-DIA73>3.0.CO;2-Y

Pickup, J. C., & Crook, M. A. (1998). Is type II diabetes mellitus a disease of the innate immune system? *Diabetologia, 41*(10), 1241-1248.

Pickup, J. C., & Mattock, M. B. (2003). Activation of the innate immune system as a predictor of cardiovascular mortality in type 2 diabetes mellitus. *Diabetic Medicine, 20*(9), 723-726. doi: 10.1046/j.1464-5491.2003.00990.x

Pickup, J. C. (2004). Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. *Diabetes Care, 27*(3), 813-823. doi: 10.2337/diacare.27.3.813

Pisani, P., Parkin, D. M., Munoz, N., & Ferlay, J. (1997). Cancer and infection: estimates of the attributable fraction in 1990. *Cancer Epidemiology, Biomarkers and Prevention, 6*(6), 387-400.

Ponikowska, B., Suchocki, T., Paleczny, B., Olesinska, M., Powierza, S., Borodulin-Nadzieja, L., Reczuch, K., Haehling, S.V., Doehner, W., Anker, S.D., Cleland, J.G., & Jankowska, E.A. Iron status and survival in diabetic patients with coronary artery disease. *Diabetes Care*, *36*, 4147–4156.

Pradhan, A. D., Manson, J. E., Rifai, N., Buring, J. E., & Ridker, P. M. (2001). C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *Journal of the American medical Association, 286*(3), 327-334. doi: 10.1001/jama.286.3.327

Prager, M., Turel, Z., Speidl, W. S., Zorn, G., Kaun, C., Niessner, A…& Wojta, J. (2002). Chlamydia pneumoniae in carotid artery atherosclerosis: a comparison of its presence in atherosclerotic plaque, healthy vessels, and circulating leukocytes from the same individuals. *Stroke, 33*(12), 2756-2761. doi: 10.1161/01.STR.0000039322.66575.77

Richardson, A. P., & Tayek, J. A. (2002). Type 2 diabetic patients may have a mild form of an injury response: a clinical research center study. *American Journal of*

Physiology, Endocrinology, and Metabolism, 282(6), E1286-1290. doi: 10.1152/ajpendo.00132.2001

Rider, J. R., Ollier, W. E., Lock, R. J., Brookes, S. T., & Pamphilon, D. H. (1997). Human cytomegalovirus infection and systemic lupus erythematosus. *Clinical and Experimental Rheumatology, 15*(4), 405-409.

Ridker, P. M., Hennekens, C. H., Stampfer, M. J., & Wang, F. (1998). Prospective study of herpes simplex virus, cytomegalovirus, and the risk of future myocardial infarction and stroke. *Circulation, 98*(25), 2796-2799. doi: 10.1161/01.CIR.98.25.2796

Ridker, P. M. (1999). Evaluating novel cardiovascular risk factors: can we better predict heart attacks? *Annals of Internal Medicine, 130*(11), 933-937. doi: 10.7326/0003-4819-130-11-199906010-00018

Ridker, P. M., Hennekens, C. H., Buring, J. E., & Rifai, N. (2000). C-Reactive Protein and Other Markers of Inflammation in the Prediction of Cardiovascular Disease in Women. *New England Journal of Medicine, 342*(12), 836-843. doi10.1056/NEJM200003233421202

Roberts, B. W., & Cech, I. (2005a). Association of type 2 diabetes mellitus and seroprevalence for cytomegalovirus. *South Med J, 98*(7), 686-692.

Roberts, B. W., & Cech, I. (2005b). Association of type 2 diabetes mellitus and seroprevalence for cytomegalovirus. *Southern Medical Journal, 98*(7), 686-692.

Roivainen, M., Viik-Kajander, M., Palosuo, T., Toivanen, P., Leinonen, M., Saikku, P., & Manttari, M. (2000). Infections, inflammation, and the risk of coronary heart disease. *Circulation, 101*(3), 252-257.

Roizman, B., Knipe, D.M., & Whiteley,R.J. (2007). *Herpes simplex viruses*. In: Knipe, D.M., Howeley, P.M. Fields Virology. (5th Ed). Lippincott Williams & Wilkins; Baltimore, MD: pp. 2503-2602.

Rosenthal, J.A. (1996). Qualitative descriptors of strength of associations and effect size. *Journal of Social Service Research, 21*(4), 37-59. doi: 10.1300/J079v21n04_02

Ross, R. (1999). Atherosclerosis--an inflammatory disease. *New England Journal of Medicine, 340*(2), 115-126. doi: 10.1056/NEJM199901143400207

Research Triangle Institute. (2013). *SUDAAN Language Manual, Volumes 1 and 2, Release 11***.** First Edition. Research Triangle Park, NC: Research Triangle Institute.

Roecker, E. B. (1991). Prediction error and its estimation for subset—selected models. *Technometrics*, *33*, 459–468.

Rupprecht, H. J., Blankenberg, S., Bickel, C., Rippin, G., Hafner, G., Prellwitz, W., & Whitley, R.J. (2001). Impact of viral and bacterial infectious burden on long-term prognosis in patients with coronary artery disease. *Circulation, 104*(1), 25-31. doi: 10.1161/hc2601.091703

Sacks, S. L., Griffiths, P. D., Corey, L., Cohen, C., Cunningham, A., & Dusheiko, G. M. (2004). HSV-2 transmission. *Antiviral Research, 63 Suppl 1*, S27-35. doi:10.1016/j.antiviral.2004.06.005

Saito, I., Folsom, A. R., Brancati, F. L., Duncan, B. B., Chambless, L. E., & McGovern, P. G. (2000). Nontraditional risk factors for coronary heart disease incidence among persons with diabetes: the Atherosclerosis Risk in Communities (ARIC) Study. *Annals of Internal Medicine, 133*(2), 81-91. doi:10.7326/0003-4819-133-2-200007180-00021

Samandary, S., Kridane-Miledi, H., Sandoval, J.S., Choudhury, Z., Langa-Vives, F., Spencer, D… & BenMohamed, L. (2014). Associations of HLA-A, HLA-B and HLA-C alleles frequency with prevalence of herpes simplex virus infections and diseases across global populations: implication for the development of an universal CD8+ T-cell epitope-based vaccine. *Human Immunology, 75*(8), 715-729. doi:10.1016/j.humimm.2014.04.016

Sandri-Goldin, R. M. (2006). *Alpha herpeviruses: molecular and celular biology*. Irvine, CA: Caister Academic Press. SAS Institute Inc., SAS 9.1.3 Help and Documentation, Cary, NC: SAS Institute Inc., 2000-2004.

Sawtell, N. M., Thompson, R. L., & Haas, R. L. (2006). Herpes simplex virus DNA synthesis is not a decisive regulatory event in the initiation of lytic viral protein expression in neurons in vivo during primary infection or reactivation from latency. *Journal of Virology, 80*(1), 38-50. doi: 10.1128/JVI.80.1.38-50.2006

Schacker, T., Ryncarz, A. J., Goddard, J., Diem, K., Shaughnessy, M., & Corey, L. (1998). Frequent recovery of HIV-1 from genital herpes simplex virus lesions in HIV-1-infected men. *Journal of American Medical Association, 280*(1), 61-66. doi:10.1001/jama.280.1.61

Schlipköter, U., & Flahault, A. (2010). Communicable diseases: achievements and challenges for public health. *Public Health Reviews, 32*:90-119.

Schmidt, M. I., Duncan, B. B., Sharrett, A. R., Lindberg, G., Savage, P. J., Offenbacher, S. & Heiss, G. (1999). Markers of inflammation and prediction of diabetes mellitus in adults (Atherosclerosis Risk in Communities study): a cohort study. *Lancet, 353*(9165), 1649-1652. doi: 10.1016/S0140-6736(99)01046-6

Sethi, S., Wrona, C., Eschberger, K., Lobbins, P., Cai, X., & Murphy, T. F. (2008). Inflammatory profile of new bacterial strain exacerbations of chronic obstructive pulmonary disease. *American Journal of Respiratory and Critical Care Medicine, 177*(5), 491-497. doi: 10.1164/rccm.200708-1234OC

Sfriso, P., Ghirardello, A., Botsios, C., Tonon, M., Zen, M., Bassi, N., Bassetto, F., & Doria, A. (2010). Infections and autoimmunity: the multifaceted relationship. *Journal of Leukocyte Biology*, 87(3), 385-95. doi: 10.1189/jlb.0709517

Shah, B.R., & Hux, J.E. (2003). Quantifying the risk of infectious diseases for people with diabetes. *Diabetes care, 26*(2), 510-513.

Shima, K., Kuhlenbaumer, G., & Rupp, J. (2010). Chlamydia pneumoniae infection and Alzheimer's disease: a connection to remember? *Medical Microbiology and Immunology, 199*(4), 283-289. doi: 10.1007/s00430-010-0162-1

Shimomura, Y. (2008). [Herpes simplex virus latency, reactivation, and a new antiviral therapy for herpetic keratitis]. *Nihon Ganka Gakkai Zasshi, 112*(3), 247-264.

Sicree,R.A., Zimmet, P.Z., Dunstan, D.W., Cameron, A.J., Welborn, T.A., Shaw, J.E. (2008) Differences in height explain gender differences in the response to the oral glucose tolerance test- the AusDiab study. *Diabetic Medicine,* 25: 296-302.

Simanek, A.M., Dowd, J.B., & Aiello, A.E. (2009). Persistent pathogens linking socioeconomic position and cardiovascular disease in the US. International Journal of Epidemiology, 38(3), 775-787, doi: 10.1093/ije/dyn273

Smith, J. S., & Robinson, N. J. (2002). Age-specific prevalence of infection with herpes simplex virus types 2 and 1: a global review. *Journal of Infectious Diseases, 186 Suppl 1,* S3-28. doi: 10.1086/343739

Snijder, M. B., Dekker, J. M., Visser, M., Stehouwer, C. D., Yudkin, J. S., Bouter, L. M… & Seidell, J.C. (2003). Prospective relation of C-reactive protein with type 2 diabetes: response to Han et al. *Diabetes Care, 26*(5), 1656-1657; author reply 1657-1658. doi: 10.2337/diacare.26.5.1656

Sobngwi, E., Choukem, S.P., Agbalika, F., Blondeau, B., Fetita, L.S., Lebbe, C… & Gautier, J.F. (2008). Ketosis-prone type 2 diabetes mellitus and human herpesvirus 8 infection in sub-Saharan Africans. *Journal of American Medical Association, 299*(23), 2770-2776. doi: 10.1001/jama.299.23.2770

Soderberg-Naucler, C. (2006). Does cytomegalovirus play a causative role in the development of various inflammatory diseases and cancer? *Journal of Internal Medicine, 259*(3), 219-246. doi: 10.1111/j.1365-2796.2006.01618.x

SoRelle, R. (1998). Chronic disease: infectious cause? *Circulation,* 97(25), 2481.

Sorlie, P. D., Adam, E., Melnick, S. L., Folsom, A., Skelton, T., Chambless, L. E. & Melnick, J.L. (1994). Cytomegalovirus/herpesvirus and carotid atherosclerosis: the ARIC Study. *Journal of Medical Virology, 42*(1), 33-37.

Sorlie, P.D., Nieto, F.J., Adam, E., Folsom, A.R., Shahar, E., & Massing, M. (2000). A prospective study of cytomegalovirus, herpes simplex virus 1, and coronary heart disease – The atherosclerosis risk in communities (ARIC) study. *Archives of Internal Medicine, 160*(13), 2027-2032.

Spranger, J., Kroke, A., Mohlig, M., Hoffmann, K., Bergmann, M. M., Ristow, M., Boeing, H., & Pfeiffer, A.F.H. (2003). Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. *Diabetes, 52*(3), 812-817. doi:10.2337/diabetes.52.3.812

Sriharan, M., Reichelt, A. J., Opperman, M. L., Duncan, B. B., Mengue, S. S., Crook, M. A., & Schmidt, M.I. (2002). Total sialic acid and associated elements of the metabolic syndrome in women with and without previous gestational diabetes. *Diabetes Care, 25*(8), 1331-1335. doi:10.2337/diacare.25.8.1331

Stanberry, L. R., Cunningham, A. L., Mindel, A., Scott, L. L., Spruance, S. L., Aoki, F. Y., & Lacey, C.J. (2000). Prospects for control of herpes simplex virus disease through immunization. *Clinical Infectious Diseases, 30*(3), 549-566. doi: 10.1086/313687

Stanberry, L. R., & Rosenthal, S. L. (2002). Genital herpes simplex virus infection in the adolescent: special considerations for management. *Pediatric Drugs, 4*(5), 291-297.

Staras, S.A., Dollard, S.C., Radford, K.W., Flanders, W.D. Pass, R.F. & Cannon, M.J. (2006). Seroprevalence of cytomegalovirus infection in the U.S., 1988-1994. *Clinical Infectious Diseases, 43*(9), 1143-1151

Steiner, P. G. K. (1995). Herpes simplex virus latent infection in nervous system. *Neurovirology, 1,* 19-29.

Stevens, J. G. (1989). Human herpesviruses: a consideration of the latent state. *Microbiology Reviews, 53*(3), 318-332.

Stuart, P. M., Summers, B., Morris, J. E., Morrison, L. A., & Leib, D. A. (2004). CD8(+) T cells control corneal disease following ocular infection with herpes simplex virus type 1. *Journal of General Virology, 85*(Pt 7), 2055-2063. doi: 10.1099/vir.0.80049-0

Sun, Y., Pei, W., Wu, Y., Jing, Z., Zhang, J., & Wang, G. (2004). Herpes simplex virus type 2 infection is a risk factor for hypertension. *Hypertension Research, 27*(8), 541-544. doi: 10.1291/hypres.27.541

Swanborg, R. H., Whittum-Hudson, J. A., & Hudson, A. P. (2003). Infectious agents and multiple sclerosis--are Chlamydia pneumoniae and human herpes virus 6 involved? *Journal of Neuroimmunology, 136*(1-2), 1-8. doi: 10.1016/S0165-5728(02)00465-4

Szucs, T. D., Berger, K., Fisman, D. N., & Harbarth, S. (2001). The estimated economic burden of genital herpes in the United States. An analysis using two costing approaches. *BMC Infectious Diseases, 1*, 5. doi: 10.1186/1471-2334-1-5

Taylor, T. J., Brockman, M. A., McNamee, E. E., & Knipe, D. M. (2002). Herpes simplex virus. *Frontiers in Biosciene, 7*:d752-764.

Temelkova-Kurktschiev, T., Henkel, E., Koehler, C., Karrei, K., & Hanefeld, M. (2002). Subclinical inflammation in newly detected Type II diabetes and impaired glucose tolerance. *Diabetologia, 45*(1), 151. doi10.1007/s001250200017

Tesfaye, S., Cullen, D.R., Wilson, R.M., & Woolley, P.D. (1991). Diabetic ketoacidosis precipitated by genital herpes infection. *Diabetes Research and Clinical Practice, 13*(1-2), 83-84.

Thorand, B., Lowel, H., Schneider, A., Kolb, H., Meisinger, C., Frohlich, M., & Koenig, W. (2003). C-reactive protein as a predictor for incident diabetes mellitus among middle-aged men: results from the MONICA Augsburg cohort study, 1984-1998. *Archives of Internal Medicine, 163*(1), 93-99. doi10.1001/archinte.163.1.93

Todar, T. (2009). The Microbial World. *Bacteriology at UW-Madison*, from http://textbookofbacteriology.net/adaptive_6.html

Torok-Storb, B., Fries, B., Stachel, D., & Khaira, D. (1993). Cytomegalovirus: variations in tropism and disease. *Leukemia, 7 Suppl 2*, S83-85.

van der Kleij, D., & Yazdanbakhsh, M. (2003). Control of inflammatory diseases by pathogens: lipids and the immune system. *European Journal of Immunology, 33*(11), 2953-2963. doi: 10.1002/eji.200324340

Vittinghoff, E., Glidden, D.V., Shiboski, S.C., & McCulloch, C.E. (2012). *Regression methods in biostatistics*. Linear, logistic, survival, and repeated measures models: 2nd ed. (100), 93-158. San Francisco, CA: Springer.

Vlassara, H., Cai, W., Crandall, J., Goldberg, T., Oberstein, R., Dardaine, V., Peppa, M., & Rayfield, E.J. (2002). Inflammatory mediators are induced by dietary glycotoxins, a major risk factor for diabetic angiopathy. *Proceedings of the National Academy of Sciences of the United States of America, 99*(24), 15596-15601.

Vozarova, B., Weyer, C., Lindsay, R.S., Pratley, R.E., Bogardus, C., & Tataranni, P.A. (2002). High white blood cell count is associated with a worsening of insulin sensitivity and predicts the development of type 2 diabetes. *Diabetes*. 51(2), 455-61.

Wald, A., Langenberg, A. G., Link, K., Izu, A. E., Ashley, R., Warren, T. & Corey, L. (2001). Effect of condoms on reducing the transmission of herpes simplex virus type 2 from men to women. *Journal of American Medical Association, 285*(24), 3100-3106. doi:10.1001/jama.285.24.3100

Wald, A., Corey, L. (2007). *Human herpesviruses: Biology, therapy, and immunoprophylaxis*. New York, NY: Cambridge University Press. ISBN 978-0-521-82714-0.

Wannamethee, S. G., Lowe, G. D., Whincup, P. H., Rumley, A., Walker, M., & Lennon, L. (2002). Physical activity and hemostatic and inflammatory variables in elderly men. *Circulation,105*(15), 1785-1790. doi:10.1161/01.CIR.0000016346.14762.71

Watson, J., Round, A., & Hamilton, W. (2012). Raised inflammatory markers. *British Medical Journal, 344*, e454. doi: 10.1136/bmj.e454

Watts, A., Crimmins, E. M., & Gatz, M. (2008). Inflammation as a potential mediator for the association between periodontal disease and Alzheimer's disease. *Neuropsychiatric Disease and Treatment, 4*(5), 865-876. doi10.2147/NDT.S3610

Weidinger, G., Czub, S., Neumeister, C., Harriott, P., ter Meulen, V., & Niewiesk, S. (2000). Role of CD4(+) and CD8(+) T cells in the prevention of measles virus-induced encephalitis in mice. *Journal of General Virology*, *81*(Pt 11), 2707-2713.

Whitley, R. J. (1996). *Medical Microbiology* (4th ed.). Chp 68: Herpesviruses. Galveston, TX: University of Texas Medical Branch at Galveston.

Whitley, R., Kimberlin, D. W., & Prober, C. G. (2007). Pathogenesis and disease. In *Human Herpesviruses: Biology, Therapy, and Immunoprophylaxis*. Cambridge, NY: Cambridge University Press.

Wilkinson, L., & Dallal, G.E. (1981). Tests of significance in forward selection regression with an F-to enter stopping rule. *Technometrics*, *23*, 377–380.

World Health Organization. (2012). Cardiovascular diseases. Retrieved September 15, 2012, 2012, from http://www.euro.who.int/en/what-we-do/health-topics/noncommunicable-diseases/cardiovascular-diseases/definition

World Health Organization. (2014). Serum ferritin concentrations for the assessment of iron status and iron deficiency in populations. Retrieved 11/4/2014 from http://www.who.int/vmnis/indicators/serum_ferritin.pdf

Woloshin, S., & Schwartz, L.M. (2005). Distribution of c-reactive protein values in the U.S. *New England Journal of Medicine, 352*, 1611-1613. doi: 10.1056/NEJM200504143521525

Wu, J. T., & Wu, L. L. (2006). Linking inflammation and atherogenesis: soluble markers identified for the detection of risk factors and for early risk assessment. *Clinica Chimica Acta, 366*(1-2), 74-80. doi.org/10.1016/j.cca.2005.10.016

Xu, F., Sternberg, M. R., Kottiri, B. J., McQuillan, G. M., Lee, F. K., Nahmias, A. J., & Markowitz, L.E. (2006). Trends in herpes simplex virus type 1 and type 2 seroprevalence in the United States. *The Journal of the American Medical Association, 296*(8), 964-973. doi:10.1001/jama.296.8.964.

Yarnell, J.W., Baker, I.A., Sweetnam,P.M, bainton, D., O'Brien, J.R., Whitehead, P.J., & Elwood, P.C. (1991). Fibrinogen, viscosity, and white blood cell count are major risk factors for ischemic heart disease: the caerphilly and speedwell collaborative heart disease studies. *Circulation, 83*(3), 836–844.

Yuhua, S., Yongjian, W., Weidong, P., & Yuejin, Y. (2005). An association of herpes simplex virus type 1 infection with type 2 diabetes. *Diabetes Care, 28*(2), 435-436. doi: 10.2337/diacare.28.2.435.

Zhou, Y. F., Leon, M. B., Waclawiw, M. A., Popma, J. J., Yu, Z. X., Finkel, T., & Epstein, S.E. (1996). Association between prior cytomegalovirus infection and the risk of restenosis after coronary atherectomy. *New England Journal of Medicine, 335*(9), 624-630. doi: 10.1056/NEJM199608293350903

Zhu, J., Quyyumi, A., Norman, J.E., Csako, G., Waclawiw, M.A., Shearer, G.M., & Epstein, S.E. (1999). Effects of total pathogens burden on coronary artery disease risk and C-reactive protein levels. *American Journal of Cardiology, 85*(2), 140-146. doi: http://dx..org/10.1016/S0002-9149 (99)00653-0

Zhu, J., Hladik, F., Woodward, A., Klock, A., Peng, T., Johnston, C…& Koelle, D.M., Wald, A., Corey, L. (2009). Persistence of HIV-1 receptor-positive cells after HSV-2 reactivation is a potential mechanism for increased HIV-1 acquisition. *Nature Medicine, 15*(8), 886-892. doi: 10.1038/nm.2006

Zhu, J., Nieto, F. J., Horne, B. D., Anderson, J. L., Muhlestein, J. B., & Epstein, S. E. (2001). Prospective study of pathogen burden and risk of myocardial infarction or death. *Circulation, 103*(1), 45-51. doi: 10.1161/01.CIR.103.1.45

Ziegler, D. (2005). Type 2 diabetes as an inflammatory cardiovascular disorder. *Current Molecular Medicine, 5*(3), 309-322.

Zur Hausen, H. (2009). The search for infectious causes of human cancers: where and why. *Virology, 392*(1), 1-10. doi: 10.1016/j.virol.2009.06.001

Appendix A: Authorization to Use _____ From Dr. Pickup

Original E-mail

From : "Pickup, John" [john.pickup@kcl.ac.uk]

Date : 06/28/2013 03:26 AM

To : Margarita De la Cruz [margarita.delacruz@waldenu.edu]

Subject : RE: Permission to use copyright work

Dear Margarita

I am very happy to grant you permission to reproduce a figure from my paper in Diabetes Care "Inflammation and Activated Innate Immunity in the Pathogenesis of Type 2Diabetes".

With kind regards

John Pickup
Professor of Diabetes and Metabolism
King's College London School of Medicine
Hodgkin Building 2.92
Guy's Hospital Campus
London SE1 1UL, UK
Tel +44 (0)20 7848 6024
E-mail john.pickup@kcl.ac.uk

From: Margarita De la Cruz [mailto:margarita.delacruz@waldenu.edu]
Sent: 27 June 2013 7:08 PM
To: Pickup, John
Subject: Permissionto use copyright work

Appendix B: Authorization to Use _____ From Dr. Elkon

Original E-mail

From : "Elkon, Keith B." [KElkon@medicine.washington.edu]

Date : 07/01/2013 03:10 PM

To : Margarita De la Cruz [margarita.delacruz@waldenu.edu]

CC : "Elkon, Keith" [elkon@u.washington.edu], Margarita De la Cruz [margarita.delacruz@waldenu.edu], Casali Paolo [pcasali@uci.edu]

Subject : Re: Permission to use cpy right material

Yes that is fine
Keith

_____

Original E-mail
>From : "Casali, Paolo" [pcasali@uci.edu<mailto:pcasali@uci.edu>]
Date : 06/30/2013 09:18 AM
To : "elkon@u.washington.edu<mailto:elkon@u.washington.edu>" [elkon@u.washington.edu<mailto:elkon@u.washington.edu>]
CC : Margarita De la Cruz [margarita.delacruz@waldenu.edu<mailto:margarita.delacruz@waldenu.edu>]
Subject : Re: Permission to use cpy right material

Margie,
Keith will make the decision.

Thanks,
Paolo

_____

Paolo Casali, MD, Donald L Bren Professor
of Medicine, Molecular Biology & Biochemistry
Professor of Microbiology and Molecular Genetics
Director, Institute for Immunology

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