COGNITIVE DISEASE, METABOLIC DISEASE, AND INFLAMMATION IN THE HONOLULU ASIA AGING STUDY: CONNECTING THE DOTS BETWEEN INSULIN RESISTANCE, TYPE 2 DIABETES, ALZHEIMER'S DISEASE, DEMENTIA, AND FIBRINOGEN

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Dedication

To my fiancé, Jessica, thank you for understanding and dealing with my constant high levels of stress these past three years. Without you, I would not have been able to complete this odyssey.

To my family and friends, your support and encouragement is forever remembered.

To my mom and dad, for not thinking I was crazy enough to spend three more years in school; yes, I will find a job now.

To the Healthy Hawaii Initiative Team, thank you for support starting from my Master's Candidacy, all the way through the end of my Ph.D.

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Abstract

Metabolic diseases and cognitive diseases are two of the leading causes of morbidity and mortality worldwide. As medical technology and research continues to evolve and improve, the proportion of persons over 65 years of age will continue to increase. This dissertation presents analysis aimed to understand connections between inflammation, insulin resistance, type 2 diabetes, and Alzheimer's disease/dementia. The first study looks at the relationship between insulin resistance and Alzheimer's disease/dementia. The relationship between inflammation, via fibrinogen, and insulin resistance is investigated in the second study. Finally, the third study investigates the association between fibrinogen and type 2 diabetes. This dissertation utilized data from the Honolulu Asia Aging study, a longitudinal cohort of Japanese-American men who were identified using 1960 U.S. Census data and selective service registration records from World War II.

The author found that subjects who were insulin resistant at later life had decreased odds of Alzheimer's disease and dementia. Carriers of the APOE ε4 allele had at 50% increased odds of dementia and 60% increased odds of Alzheimer's disease. Subjects with elevated fibrinogen levels at later life observed increased odds of prevalent insulin resistance and type 2 diabetes, even after adjusting for potential confounders.

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List of Abbreviations:

AD:	Alzheimer's Disease
IR:	Insulin Resistance
CRP:	C-Reactive Protein
VD:	Vascular Dementia
FAD:	Familial Dementia
SAD:	Sporadic Dementia
Aβpp:	Amyloid β precursor protein
Aβ:	Amyloid β
APOε4:	Apolipoprotein ε4
T2D:	Type 2 Diabetes
Ha1c:	Hemoglobin A1c
Mets:	Metabolic Syndrome
ATP III:	Adult Treatment Panel 3
HOMA:	HOMA Insulin Resistance Index
WC:	Waist Circumference
CSF:	Cerebral Spinal Fluid
ER:	Endoplasmic Reticulum
Aβ:	Amyloid β Oligomer
HAAS:	Honolulu Asia Aging Study
HHP:	Honolulu Heart Problem
ATP:	Adenosine Triphosphate
NFT:	Neurofibrillary tangles
IRec:	Insulin Receptor
IGF:	Insulin Growth Factor
OR:	Odds Ratio
RR:	Risk Ratio (Relative Risk)

Chapter 1

Introduction

According to a report on world population aging in 2013 prepared by the Population Division within the United Nation's Department of Economic and Social Affairs, the population of older persons, defined as persons aged 60 years and older, will triple by 2050. [1] In terms of actual numbers, that means an older persons population of 841 million in 2013 will increase to over two billion by 2050. Another startling statistic is that in 1950, thirteen percent of dependents were older persons; this percentage is projected to increase to fifty percent by 2080. [1]

In developed countries, chronic diseases account for the majority of negative health outcomes. The more prevalent diseases in those developed countries include cardiovascular diseases, cancers, and neurodegenerative diseases, of which age is the main risk factor.[2] Therefore, as the world population continues to age, the prevalence of these chronic diseases, especially neurodegenerative diseases, will only increase. Of the neurodegenerative diseases, Alzheimer's disease is by far the most prevalent.[3] Researchers have created models to estimate the increase in the prevalence of Alzheimer's disease to 80 million people worldwide by 2040 [4] and 106 million by 2050. [5]

Alzheimer's disease (AD) is often confused with dementia. The 2014 Alzheimer's Disease Facts and Figures, published by the Alzheimer's Association, defines dementia as an umbrella term for diseases and conditions characterized by a decrease in cognitive function and ability in everyday life.[6] Types of Dementia include AD, Vascular Dementia (VD) and Dementia with Lewy Bodies. Of those types of dementia, AD is the most common. [6-10] The hallmarks of AD are the presence of amyloid-β plaques between nerve cells and formations of hyperphosphorylated Tau neurofibrillary tangles within nerve cells.[7, 11, 12] Early clinical symptoms include impaired ability to recall recent events and conversations and also depression and apathy. As AD progresses in a patient, the disease causes patients to become disoriented, confused, and eventually lose their ability to speak, walk, and swallow.[6]

Alzheimer's disease is named after the German doctor Alois Alzheimer who first documented the symptoms of a patient more than 100 years ago in 1906. [6, 13] Over a century later, the exact causes and disease pathology of AD are still the subject of considerable research efforts. AD can be categorized into two types: familial AD (FAD) and sporadic AD (SAD).[11, 12, 14] Patients with FAD account for 5% of all AD cases and experience the onset of AD earlier in their lives (<65 years). The onset of FAD is caused by mutations in the genes responsible for encoding the amyloid β precursor protein (Aβpp) and presenillins 1 and 2.[11, 12, 14] The overwhelming majority (95%) of all AD cases are attributed to SAD and occur in individuals 65 years and older. Age and the presence of apolipoprotein ε4 (APOε4) are considered the main risk factors of SAD[14]. Recently, the most researched and talked about causes of SAD revolve around Type 2 Diabetes (T2D). Due to findings by some researchers, AD has been labeled as Type 3 Diabetes.[15, 16]

The hallmark of T2D is the steady decline of Beta (β) cell function, which can occur as early as 12 years before diagnosis.[17] Hyperinsulinemia, a state in the body where there is an excess of insulin circulating in the bloodstream relative to glucose, eventually results in insulin resistance (IR).[18] After consumption of a meal, glucose levels rise in the blood and the pancreas releases insulin to help move glucose from the bloodstream into the cells where it can be turned into energy in the form of ATP. Pancreatic β cells release insulin in response to the rise in glucose levels. A chronically high level of glucose will lead to hyperinsulinemia and ultimately in IR. When IR occurs, the β cells in the pancreas will increase their output of insulin to compensate for the elevated levels of glucose in the bloodstream. Eventually, the β cells will fail to produce enough insulin, resulting in the onset of T2D.[17]

The complete pathology of AD/dementia still eludes researchers. Because of the various types of dementia present, more than one hypothesis has been suggested to explain the pathology of AD/dementia. However, it is truly difficult to definitively diagnose a patient showing symptoms of cognitive impairment with dementia, VD, or AD because of the shared and similar markers of VD and AD.[19] For the past 50 years, the presence of plaques formed by amyloid-β (Aβ) and the aggregation of tau proteins to form neurofibrillary tangles (NFTs) have been thought to cause cognitive decline and memory impairment.[8, 11]

Therefore, research efforts began to focus on understanding how these plaques and NFTs are formed in hopes of elucidating the causal mechanisms and eventually finding a cure.

However, post-mortem examination of AD brains has contradicted the existing theory behind AD/dementia pathology. These examinations have shown a poor correlation between amyloid burden and pre-mortem decline.[20] Conversely, the results of the study revealed that there was a strong correlation between cognitive decline and synaptic loss. As a result, research has shifted from the formation of plaques and NFTs to the causes of synaptic loss in the brain.

The Relation of IR with AD, VD and dementia

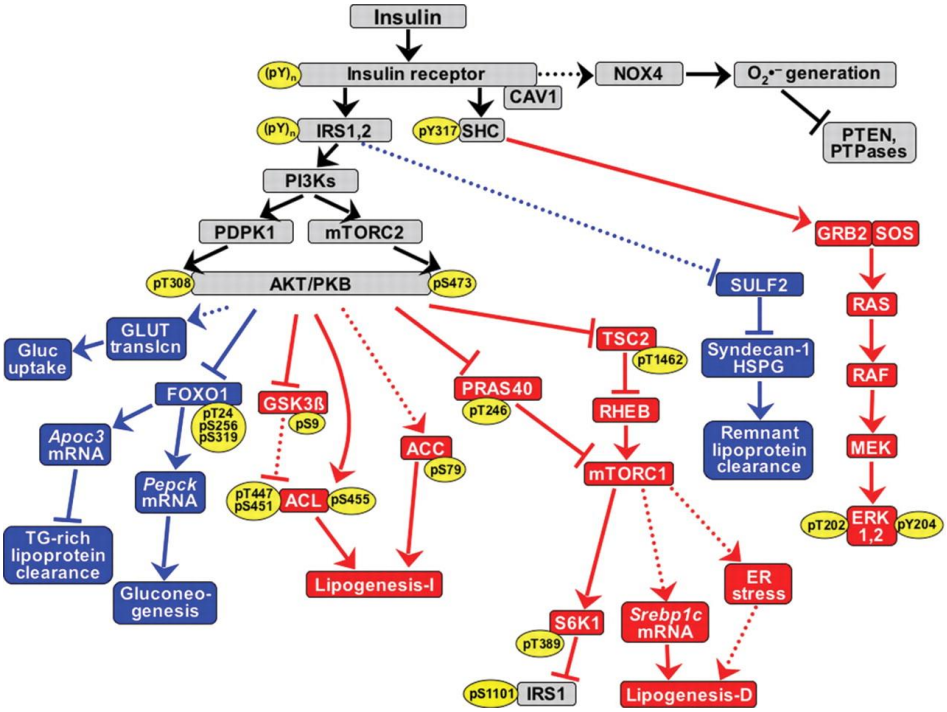
Peripherally, IR inhibits the ability of cells to maintain normal cell functions. [20, 21] Insulin is transported to the brain through the blood brain barrier via the cerebrospinal fluid (CSF). [11, 13, 14, 22] Insulin's role in the brain is linked to neuronal survival, brain function, proliferation and inhibition of neuronal apoptosis, memory formation, memory retrieval, synaptic plasticity, synaptic learning and synaptic memory.[13, 20, 21] Glucose homeostasis, energy metabolism, and white matter fiber structure and function have also been affected by insulin in the brain. [10] Insulin receptors (IRec) and insulin growth factors (IGFs) are found in large numbers throughout the brain, including the hippocampus, hypothalamus, amygdala, septum, olfactory bulb, and cerebral cortex.[9, 12, 13, 20-23]

Insulin Signaling and IR

Abundant evidence has connected insulin and insulin signaling to cognitive performance and memory in the brain, as introduced in the previous paragraphs. However, the effects of IR on insulin signaling in the brain is a relatively new field of research. From what is understood, under normal conditions, insulin binds extracellularly to the IRec, which subsequently induces autophosphorylation of the intracellular portion of the IRec.[7, 11, 13, 23] This triggers downstream, tyrosine kinase activated pathways, of which the PI3K/Akt cascade has been most studied. The activation of the PI3K/Akt cascade can initiate downstream pathways that include mTORC1, GSK3β, and the FoxO family of transcription factors.[22] mTORC1 and

FoxO downstream pathways have been shown to affect synaptic plasticity and regulate autophagy, the cell's major method of eliminated damaged organelles and misfolded proteins in neurons.[22] Figure 1 shows the complexity of insulin signaling in the brain.

Figure 1. Insulin signaling pathways in the brain



However, under insulin resistant conditions, impaired insulin signaling in the brain is most strongly thought to increase the formation of Tau NFTs, aggregation of Aβpp and Aβ, presence of oxidative stress, endoplasmic reticulum (ER) stress, and metabolic dysfunction.[7, 10, 11, 13, 20, 24] Cognitive decline and AD/dementia pathology are believed to stem from these five results of impaired insulin signaling. The serine phosphorylated GSk3β protein kinase and resulting pathways are believed to be involved in these five results along with pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), and interleukin 8 (IL-8).[23, 24]

Tau NFTs can impair axonal transport, synaptic integrity, and disrupt neuronal cytoskeletal structure and function, whereas pre-fibrillar Tau can bond to form oligomers that encourage synapse disconnection and neuronal death.[24] The formation of the NFTs are caused by a dysregulation of GSk3β due to impaired

insulin signaling. The resulting accumulation of NFTs can negatively affect cytoskeletal structure, neurite retraction, and increase synaptic disconnection.[24]

The aggregation of Aβpp and Aβ is also shown to be caused by reduced insulin signaling that again triggers increased activation of GSk3β.[11, 23] Although GSk3β is responsible for Tau NFT production, it also increases Aβ production.[8] Because the formation of Aβ plaques has been shown to be weakly correlated with cognitive decline, recent research on Aβpp and Aβ have yielded theories regarding Aβ's role in IR. Insulin is responsible for the intracellular trafficking of Aβpp and Aβ from the trans-golgi network to the plasma membrane.[13, 21] Therefore, impaired insulin signaling results in an accumulation of Aβpp and Aβ, which can form Aβ oligomers (AβOs).

AβOs play a large role in linking IR with AD/dementia pathology. AβOs have been identified as synaptotoxins and are considered the primary toxins responsible for cognitive dysfunction in AD.[20] TNF-α is a cytokine that can cause apoptosis and has also been shown to cause peripheral insulin resistance.[25] An accumulation of soluble AβOs leads to IRS1 inhibition by activating TNF-α.[25] AβOs have also been shown to instigate abnormal TNF-α/JNK pathway activation and block downstream insulin signaling.[20] TNF-α can also activate another stress kinase, IκBα (IKK), which results in the production of Aβ. Additionally, AβOs can initiate removal of insulin receptors from the cell membrane; this process not only decreases insulin signaling, but also promotes further serine phosphorylation of PI3K/Akt and GS3Kβ, resulting in increased Aβpp and Aβ production .[25] Aβpp and Aβ can also compete with insulin, disrupting insulin signaling.[24]

Metabolic dysfunction in the brain occurs when problems arise with respect to glucose utilization and ATP production. This can lead to increased oxidative stress and ER stress. IR exacerbates the effects of oxidative stress and ER stress. Consequences of oxidative stress and ER stress are the stimulation of Aβpp gene expression and cleavage that results in increased formation of Aβpp and AβOs, increased dis-inhibition of the GSK3β kinase (Tau NFT formation) and activation of pro-inflammatory networks that can increase organelle dysfunction and pro-apoptosis mechanisms.[24]

C-Reactive Protein and Inflammation

C-Reactive Protein (CRP) is an acute phase protein that plays an important part in the wound healing process and is vital to processes critical for survival.[26] CRP levels are highly elevated immediately following an acute infection or inflammatory process. However, CRP is also a marker of chronically low grade inflammation.[27, 28] CRP, a sensitive inflammatory marker, is most commonly used as a marker of cardiovascular disease.[29] CRP has been shown to strongly and independently predict diabetes and cardiovascular disease.[30] The mechanistic role CRP plays in the immunologic disease pathology of chronic diseases such as diabetes, IR and cardiovascular disease remains unclear. However, CRP will be used as a marker of inflammation for the purposes of these studies.

Fibrinogen and Inflammation

Fibrinogen is also an acute phase protein that is most commonly known for its role in clot formation. As an inflammatory marker, fibrinogen has been positively associated with cardiovascular disease, insulin resistance, and type 2 diabetes.[31-35] Mechanistically, lab studies have shown that the presence of interleukin 6 stimulates the production of fibrinogen in an IR state.[36] Similarly to CRP, the role fibrinogen plays in the pathogenesis of IR and type 2 diabetes is still unclear. For these studies, fibrinogen will also be used as a marker of inflammation.

Assessment of Insulin Resistance

Currently, the gold standard for determining IR is using the hyperinsulinemic-euglycemic clamps.[37] However, this process is expensive, time-consuming, and requires an IV infusion of insulin and multiple blood draws. As a result, multiple methods have been devised as proxies for the gold standard. Two of these methods include the HOMA-IR index and the McAuley index. Both indices require the use of fasting insulin. HOMA index also requires fasting blood glucose whereas the McAuley index requires fasting triglycerides for the calculations.

Research questions

The aim of this dissertation is to investigate three research questions: 1) Estimate the association (Odd's Ratio) between insulin resistance and dementia and Alzheimer's disease; 2) Estimate the association (Odd's Ratio) between fibrinogen and insulin resistance; 3) Estimate the association (Odd's Ratio) between fibrinogen and type 2 diabetes.

The analysis in this dissertation will use Honolulu Asia Aging Study (HAAS) data. According to Kuakini Health System's website, the "Honolulu Asia Aging Study is an outgrowth of the Kuakini Honolulu Heart Program." Participants of the Honolulu Heart Program (HHP) were identified using selective service registration records from World War 2.[38] The goal of the HAAS, established in 1991, is to better understand dementia. The HHP first started gathering data at the first examination from 1965 to 1968. To present, an additional 11 examinations have been conducted. This proposal will focus on data gathered at examinations 1, 2, 4, and 5.

Hypotheses

Paper 1: Insulin resistance will result in increased risks of AD/dementia.

Paper 2: Increased fibrinogen levels will result in increased odds of insulin resistance.

Paper 3: Increased fibrinogen levels will result in increased odds of type 2 diabetes.

Chapter 2

Examining the Relationship between Insulin Resistance and Alzheimer's Disease/Dementia

Introduction

According to a 2013 report on world population prepared by the United Nation's Population Division, the population of persons aged 60 years and older will triple from 841 million in 2013, to over two billion by 2050.[1] Alzheimer's disease (AD) is one of the leading neurologic diseases diagnosed in older adults and is the most common form of dementia.[6] Projected prevalence of worldwide AD ranges from 84 million by 2040 to over 100 million by 2050.[5, 39] Type 2 Diabetes (T2D) has been positively associated with an increased risk of dementia as well as AD,[40-43] even to the point that AD is considered Type 3 diabetes.[44]

The mechanisms through which T2D may increase the risk of dementia and AD are still up for debate. One of the most studied mechanisms is insulin's role in neuronal signaling. Insulin receptors (IRec) and insulin growth factors (IGFs) are found in large numbers in parts of the brain responsible for memory, such as the hippocampus, hypothalamus, and cerebral cortex.[21, 22] Insulin signaling is responsible for neuronal survival, memory formation, memory retrieval, synaptic plasticity and learning.[20]

It is widely known that insulin resistance (IR) is a precursor to T2D.[45] Although current research focuses on finding relationships between AD and T2D, very few studies exist that look at IR and AD or dementia. Two longitudinal studies found that midlife IR was associated with a small increased risk of AD.[46, 47] The relationship between later life IR and AD has not yet been examined. The Honolulu-Asia Aging Study (HAAS) allows for examination of the association of later life IR and AD. The objective of this study is to examine the association of later life IR and AD/Dementia in the HAAS with the hypothesis that later life IR results in increased risk of AD/Dementia.

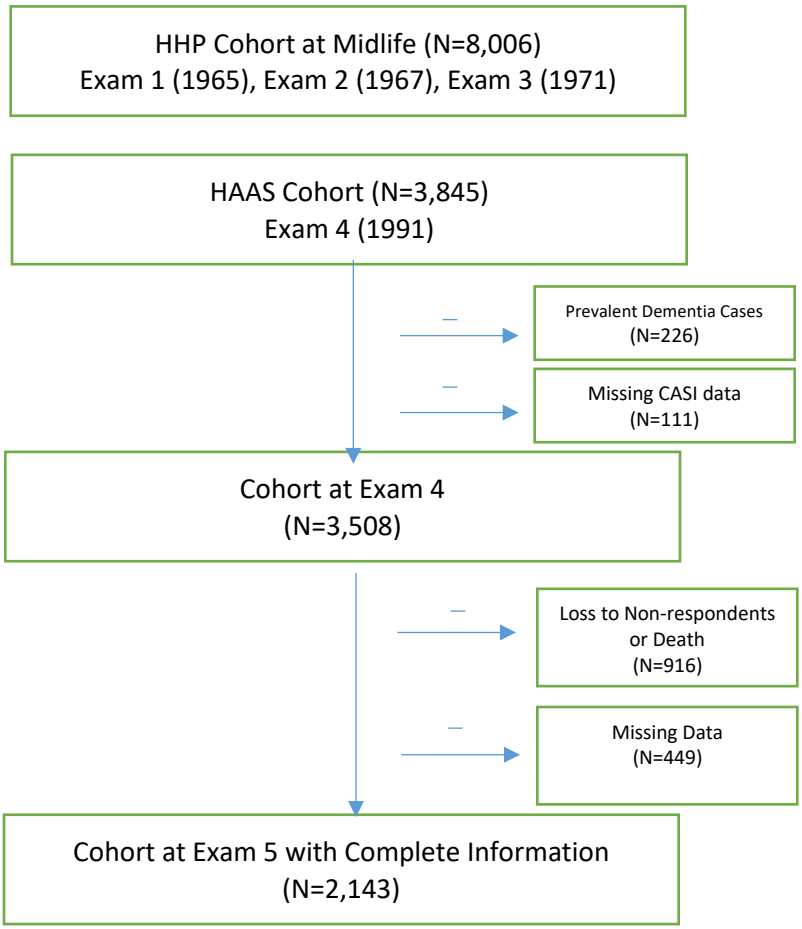
Methods

Study Population

The Honolulu Heart Program (HHP) is a longitudinal study funded by the National Heart, Lung, and Blood Institute that focuses on heart disease and stroke in a cohort of 8,006 Japanese-American men born between 1900 and 1919, who were living in Oahu at the time of the baseline examination in 1965. Participants were identified using World War II selective service records. Two subsequent examinations followed the baseline examination in 1967-1970 and 1971-1975.[48]

In 1991, the HAAS was established to begin research on risk factors associated with aging and neurodegenerative disorders. Baseline dementia status was established at the fourth examination (prevalent phase) on 3,845 surviving individuals.

Figure 2. Selection process of the cohort for the insulin resistance-AD/dementia study



The Institutional Review Board (IRB) of the University of Hawaii at Mānoa and the IRB of Kuakini Medical Center approved this study.

Diagnosis of Dementia and AD

The 100 point Cognitive Abilities Screening Instrument (CASI) was administered to all subjects in the fourth examination and all subsequent examinations to identify dementia cases.[49] The CASI is a well-recognized instrument and has been validated among Western and Japanese sample populations.[50] In exam four, CASI score and age determined a subgroup of participants for further dementia evaluation. During the fifth examination, an education-adjusted cutoff (79 for those with high education and 77 for those with low education) or an absolute drop of ≥9 points were applied to identify subjects to undergo a specific dementia examination.[51] Participants requiring further dementia diagnosis underwent clinical assessments that included detailed neuropsychological assessment, a proxy interview, and neuroimaging. A consensus committee, consisting of the study's neurologist and at least two other physicians with expertise in geriatric medicine and dementia was responsible for the final diagnosis of dementia.

Dementia was diagnosed using the DSM-III criteria.[38] Criteria from the National Institute of Neurological and Communicative Disorders and Stroke, and the Alzheimer's Disease and Related Disorders Association were used to diagnose all possible and probable AD cases. [52] For the analysis, dementia was grouped as total dementia (included all causes of dementia); and AD, with CVD and without CVD. Based on established neuropathological criteria[53], 65% of clinical AD cases met the criteria for definite or probable AD.[54]

Assessment of Insulin Resistance

Insulin resistance was estimated using the Homeostatic Model Assessment (HOMA) and the McAuley index. HOMA-IR index was calculated as [fasting insulin (μU/mL) x fasting blood glucose (mg/dL) / 405].[55] Serum glucose was used instead of fasting blood glucose but should have a minimal effect on the outcome of HOMA.[56] McAuley index was calculated as exp[2.63- 0.28 x ln (fasting

insulin (μU/ml))- 0.31 x ln (fasting triglycerides (mmol/l))].[57] The Japan Diabetes Society recommended HOMA values ≥2.5 as identifying IR and this cutoff has been used in previous Asian Studies; and a cut off of ≤5.8 based off the McAuley index was used.[57, 58] In addition to the McAuley index and HOMA-IR index, a third variable was created to indicate IR. Subjects who were considered IR by both the McAuley and HOMA indices were then considered IR in the Combined index. All other subjects in the Combined Index were considered non-IR.

Measure of Confounders and Modifiers

APOE ε4 allele has been shown to be an effect modifier in the relationship between IR and dementia and AD in Japanese cohorts.[47, 59] APOE ε4 allele genotyping was performed using PCR amplification, following the method of Hixson and Vernier.[60] Participants with at least one copy of the ε4 allele were categorized as APOE ε4 allele positive. Those with genotype ε2ε4 were excluded from the analysis due to possible opposing effect of ε2 and ε4 alleles on dementia.[61]

Midlife age, later life hypertension[62-64], midlife BMI[65-67], later life smoking[68, 69], later life coronary heart disease[70-72], later life prevalent type 2 diabetes[38], and difference in total cholesterol[73] are possible confounders in this study. Hypertension, CHD, and type 2 diabetes were also identified as possible effect modifiers. Midlife BMI, smoking, and age were used because midlife variables are less likely to be influenced by preclinical dementia status. Prevalent hypertension was defined as systolic blood pressure ≥140 mm HG, diastolic blood pressure ≥90 mm HG or use of hypertensive medication.[74] Smoking status was self-reported and categorized by never, past, or current smoker. An Autoanalyzer 1 N24B cholesterol method was used to determine total cholesterol values.[48] Stewart et al. found that a decrease in total serum cholesterol from midlife to later life is associated with dementia and AD.[73] Therefore, change in total cholesterol is represented by the difference in Exam 4 and Exam 1 total cholesterol values. BMI (kg/m^2) at Exam 2 was calculated from participant's height and weight. Self-report of doctor's diagnosis, use of insulin intake or oral hypoglycemic medications was used to determine prevalent diabetes status at exam 4.[38]

Alcohol was measured in ounces per month consumed, then recoded into nondrinker, < 1 drink a day (up to 3 ounces per month), 1-2 drinks per day (3 to 30 ounces per month) and ≥3 drinks per day.[75] Published results are mixed regarding the relationship between alcohol consumption and AD. Later life alcohol consumption has been shown to increase the risk of AD[76, 77] while the findings on mid-life alcohol consumption and risk of AD are still inconclusive.[78, 79] Mid-life alcohol consumption has been shown to increase insulin sensitivity.[80, 81] A study conducted within the HAAS confirms a U shaped relationship between alcohol and cognitive performance.[75]

Statistical Analysis

Statistical analysis was conducted using SAS software version 9 (SAS Institute, Cary, NC). Univariate analysis included comparing cohort characteristics between IR and non-IR subjects using Pearson Chi Square test for categorical variables or t-test for continuous variables. Pearson correlation coefficient was calculated for HOMA Index and McAuley Index. Insulin Resistance was coded IR or not. Incidence dementia and AD cases from exam 5 and exam 6 were included for this analysis. Because time at risk was not calculated, odds ratios for dementia and AD associated with later life IR was estimated by logistic regression. Dementia and AD are considered rare diseases, therefore the odds ratios will be used to estimate the risk of disease by IR status. Logistic regression analyses were performed as crude and adjusted for possible confounders.

In addition to the unadjusted model, two adjusted models were examined. The first model includes possible confounders: midlife BMI, midlife Age, later life alcohol, midlife smoking, and change in total cholesterol. The second model includes all potential confounders from model 1 with the addition of additional possible confounders and possible modifiers: APOE ε4 allele status, prevalent hypertension, prevalent CHD, and prevalent type 2 diabetes. In order to examine possible effect modification, I stratified by APOE ε4 allele status, prevalent hypertensive status, prevalent coronary heart disease status, and prevalent type 2 diabetes status.

Results

The baseline characteristics of study population are shown in Table 1. Of the 2,143 subjects with complete information at baseline, a total of 198 developed dementia, of which 116 were AD. Subjects divided by HOMA quartiles significantly differed by age and BMI; had differences in smoking history; differed by prevalent CHD, prevalent hypertension, and prevalent diabetes statuses. Differences between McAuley index quartiles reflected those found using HOMA index. However, differences by McAuley index quartiles also were found in incident dementia cases. The correlation between HOMA index and McAuley index can be seen in Figure 3, where r= -0.60 (p-value <0.0001).

In crude logistic regression models, subjects who were IR using the McAuley and Combined Indices had significantly lower odds for AD than subjects who were not IR(Table 2). Similarly, IR subjects determined by McAuley and Combined indices were also at a decreased odds of dementia (Table 3). After adjustment for potential confounders, the observed inverse association between IR and dementia remained for both McAuley and combined indices. However, the addition of potential confounders attenuated the significant OR between IR and AD. Using the Combined Index, subjects with the APOE ε4 allele had higher odds of AD (OR 1.61 95% CI 1.02 – 1.20) and dementia (OR 1.51 95% CI 1.06 - 2.17). Midlife age at Exam 1 was also significant using the Combined Index for both AD/dementia (OR 1.15 95% CI 1.10 - 1.18).

Stratification by APOE ε4 allele, prevalent hypertension, prevalent CHD, and prevalent type 2 diabetes did not yield any statistically significant results when looking at the relationship between IR and AD/dementia (Tables 4 and 5).

Discussion

This is the first study that looks at possibility of later-life IR as a risk factor for AD, using two distinct insulin resistance indices. Our data shows an inverse relationship between IR and AD, using either of the two IR indices. These results conflict with a previous study in the HAAS that looked at fasting insulin and incident dementia where a U shaped distribution of dementia cases by fasting insulin level was reported.[82] However, subjects with extreme levels of fasting insulin were included in that study, which may have contributed to the right side of their U shaped curve. Consequently, all subjects with extreme fasting

insulin levels were eliminated from our study cohort. Our findings did not show any non-linear association between IR and AD or dementia.

Studies investigating the association between IR and AD are less common. A cross-sectional study conducted in Hisayama, Japan found that IR subjects had a 64% increased odds of having neuritic plaques [83]while another cross-sectional study showed greater odds of AD in subjects with higher fasting insulin levels.[84] Three longitudinal studies looking at IR and AD had mixed results. Subjects with IR in the Rotterdam Study had a higher risk of AD up to three years after baseline. However, that association no longer remained in subsequent examinations after 3 years.[46] The Uppsala Longitudinal Study of Adult Men found that IR was associated with AD only in subjects with the APOE ε4 allele.[47] The Baltimore Longitudinal Study of Aging utilized autopsies to identify AD cases and no association was found between HOMA and AD.[85]

Our findings of an inverse relationship between IR and AD may explained by a couple of theories. It is widely accepted that the brain houses many IRec and IGF. Brain insulin signaling results in autophosphorylation of the IRec and triggers downstream tyrosine kinase pathways which have been shown to affect synaptic plasticity.[22] Impaired insulin signaling in the brain is most strongly thought to affect the formation of Tau NFTs, aggregation of Aβpp and Aβ, presence of oxidative stress, endoplasmic reticulum (ER) stress, and metabolic dysfunction.[7, 10, 11, 13, 20, 24, 86] However, these conditions exist after many years of IR conditions. Exams 4 and 5 were separated by 3 years, which may prove to be insufficient time for IR to negatively affect insulin signaling in the brain. But this short term state of hyperinsulinemia can provide a short term positive effect on memory and brain function. Insulin signaling in the brain declines with advanced age.[87] Craft et al. showed increased performance in memory tests in slightly demented AD patients in an induced hyperinsulinemia state through intravenous injection of insulin.[88] Intra-nasal administration of insulin has also resulted in significant memory improvement.[89] This suggested protective effect of hyperinsulinemia might explain why our IR subjects who were free of diabetes had an even lower OR of dementia and AD.

Another explanation is the measurement of IR during mid-life versus late-life. Similar situations with mid and late life cholesterol, blood pressure, and BMI occur where higher later life levels of those three risk factors are actually protective for AD/dementia.[90, 91] hyperinsulinemia may actually be an indicator of better health status. Fujita et al. showed hyperinsulinemia is necessary to stimulate skeletal muscle protein anabolism in the elderly.[92] Similarly, elevated blood pressure at midlife is considered a risk factor for dementia, yet blood pressure was lower in demented patients in older life than non-demented patients.[63]

Our subjects with the APOE ε4 allele had a 50% increase in odds of dementia and AD, confirming results from previous studies that carriers of the APOE ε4 allele are at greater risk of dementia and AD.[93, 94] After stratification by APOE ε4, IR subjects who had the APOE ε4 allele had slightly lower odds ratios for AD, but higher for dementia, than subjects who did not have the APOE ε4 allele. However both OR were still less than 1 and not significant. IR has been shown to be associated with AD in non-carriers of APOE ε4.[95] The existence of contradictory results show that further research is needed.[38, 47, 96] Recent studies are showing a lack of interaction between IR and the APOE ε4 allele. Ragogna et al. concluded no relationship between IR, using multiple IR indices, and APOE ε4. Schrijvers et al. did not find a multiplicative nor an additive interaction between HOMA and APOE ε4, and a study by Willette et al. failed to find significant interaction between HOMA and the APOE ε4 allele.[46, 97]

This study has some important strengths. First, the HAAS was restricted to men of the same age and ethnicity, which reduced confounding by age, disease, sex-related factors and genetic factors. While recall bias is still possible, the longitudinal nature of this study decreases the chances of recall bias. Unlike previous studies that used HOMA and fasting insulin as indicators of IR, this study utilized the McAuley index. The McAuley index is the IR index best suited for epidemiological studies and this was the first use of McAuley index to examine IR and AD/dementia in a Japanese population.[58] While HOMA and fasting insulin are easily calculated, those indices do not account for triglyceride levels. Elevated triglycerides is one of the criterion for the Metabolic Syndrome (MetS), and MetS is associated with increased risk of AD.[98, 99] The McAuley index is the only IR index that uses triglyceride levels, and accounts for the effect of triglycerides on AD. Additionally, the use of the Combined index allowed for greater sensitivity in identifying IR subjects.

Some limitations exist in this study. Unfortunately, repeated measurements of IR were not available during subsequent cognitive examinations. It is possible for subjects with IR to revert back to normal levels, which may affect the odds estimates. Also, we lacked the samples needed to study midlife effects of IR on AD to confirm whether a difference exists between midlife IR and later-life IR on AD. Although recall bias was greatly minimized due to IR classification using results from laboratory testing, recall bias is still possible regarding alcohol and smoking history. The use of midlife smoking status limits the effect of non-differential misclassification of AD/dementia. Misclassification of alcohol consumption at exam 4 would also be non-differential with respect to AD/dementia status. While the restrictions by age, ethnicity and gender reduces confounding, this also limits the generalizability of this study. Due to recruitment and identification of participants using Census data and Selective Service registration records, selection bias is a possibility. Loss to follow up is also another source of bias. However, the loss to follow up by IR status seems to be random, which would result in non-differential misclassification and suggest an even stronger association.

Further studies are required to elucidate the relationship between late-life IR and AD/dementia. In the future, the use of multiple IR indices, including the McAuley index, may provide increased accuracy in determining IR status.

Table 1. Selected characteristics of Participants by Insulin Resistance status determined by HOMA index, McAuley index, and the Combined index: The Honolulu-Asia Aging Study

Variables		HOMA			McAuley			Combined		
		No	Yes	P-Value	No	Yes	P-Value	No	Yes	P-Value
N=2,173		666 (30.62%)	1,477 (67.98%)	N/A	1,217 (55.95%)	926 (42.257%)	N/A	1,248 (57.38%)	895 (41.15%)	N/A
Age (years)		77.43 ± 3.90	76.82 ± 3.73	0.0005	77.28 ± 3.92	76.66 ± 3.58	0.0002	77.25 ± 3.91	76.68 ± 3.60	0.0007
APOE ε4	No	551 (25.71%)	1205 (56.23%)	0.52	1014 (47.32%)	742 (34.62%)	0.057	1042 (48.62%)	714 (33.32%)	0.03
	Yes	115 (5.37%)	272 (12.69%)		203 (9.47%)	184 (8.59%)		206 (9.61%)	181 (8.45%)	
BMI at Exam 2		22.73 ± 2.65	24.43 ± 2.66	<0.0001	23.38 ± 2.74	24.59 ± 2.66	<0.0001	23.37 ± 2.74	24.65 ± 2.64	<0.0001
Prevalent Hypertension	No	225 (10.50%)	317 (14.79%)	<0.0001	369 (17.22%)	173 (8.07%)	<0.0001	376 (17.55%)	166 (7.75%)	<0.0001
	Yes	441 (20.58%)	116 (54.13%)		848 (39.57%)	753 (35.14%)		872 (40.69%)	729 (34.02%)	
Prevalent CHD	No	569 (26.55%)	1163 (54.27%)	0.0003	1007 (46.99%)	725 (33.83%)	0.0095	1037 (48.39%)	695 (32.43%)	0.0016
	Yes	97 (4.53%)	314 (14.65%)		210 (9.80%)	201 (9.38%)		211 (9.85%)	200 (9.33%)	
Change in Total Cholesterol (mg/dL)				0.20	25.37 ± 35.44	26.18 ± 37.99	0.61	25.35 ± 35.6	26.24 ± 37.83	0.58
Smoking	Never	269 (12.55%)	525 (24.50%)	0.059	486 (22.68%)	308 (14.37%)	<0.0001	496 (23.15%)	298 (13.94%)	<0.0001
	Past	212 (9.89%)	479 (22.35%)		402 (18.76%)	289 (13.49%)		415 (19.37%)	276 (12.88%)	
	Current	185 (8.63%)	473 (22.07%)		329 (15.35%)	329 (15.35%)		337 (15.73%)	321 (14.98%)	
Alcohol (Exam 4)	Non-drinker	263 (12.27%)	632 (29.49%)	0.43	504 (23.52%)	391 (18.55%)	0.80	516 (24.08%)	379 (17.69%)	0.84
	< 1 a day	60 (2.80%)	133 (9.21%)		111 (5.18%)	82 (3.83%)		113 (5.27%)	80 (3.73%)	
	1 to 2 a day	228 (10.64%)	457 (21.33%)		398 (18.57%)	287 (13.39%)		408 (19.04%)	277 (12.93%)	
	≥3 a day	115 (5.37%)	255 (11.90%)		204 (9.52%)	166 (7.75%)		211 (9.85%)	159 (7.42%)	
Incident Dementia	No	598 (27.90%)	1347 (62.86%)	0.30	1087 (50.72%)	858 (40.04%)	0.0082	1118 (52.17%)	827 (38.59%)	0.026
	Yes	68 (3.17%)	130 (6.07%)		130 (6.07%)	68 (3.17%)		130 (6.07%)	68 (3.17%)	
Incident AD	No	625 (29.16%)	1402 (65.42%)	0.31	1139 (53.15%)	888 (41.44%)	0.021	1170 (54.60%)	857 (39.99%)	0.04
	Yes	41 (1.91%)	75 (3.50%)		78 (3.64%)	38 (1.77%)		78 (3.64%)	38 (1.77%)	
Prevalent Diabetes	No	594 (27.72%)	953 (44.47%)	<0.0001	968 (45.17%)	579 (27.02%)	<0.0001	996 (46.48%)	551 (25.71%)	<0.0001
	Yes	72 (3.36%)	524 (24.45%)		249 (11.62%)	347 (16.19%)		252 (11.76%)	344 (16.05%)	

Figure 3. Correlation between HOMA index and the McAuley Index

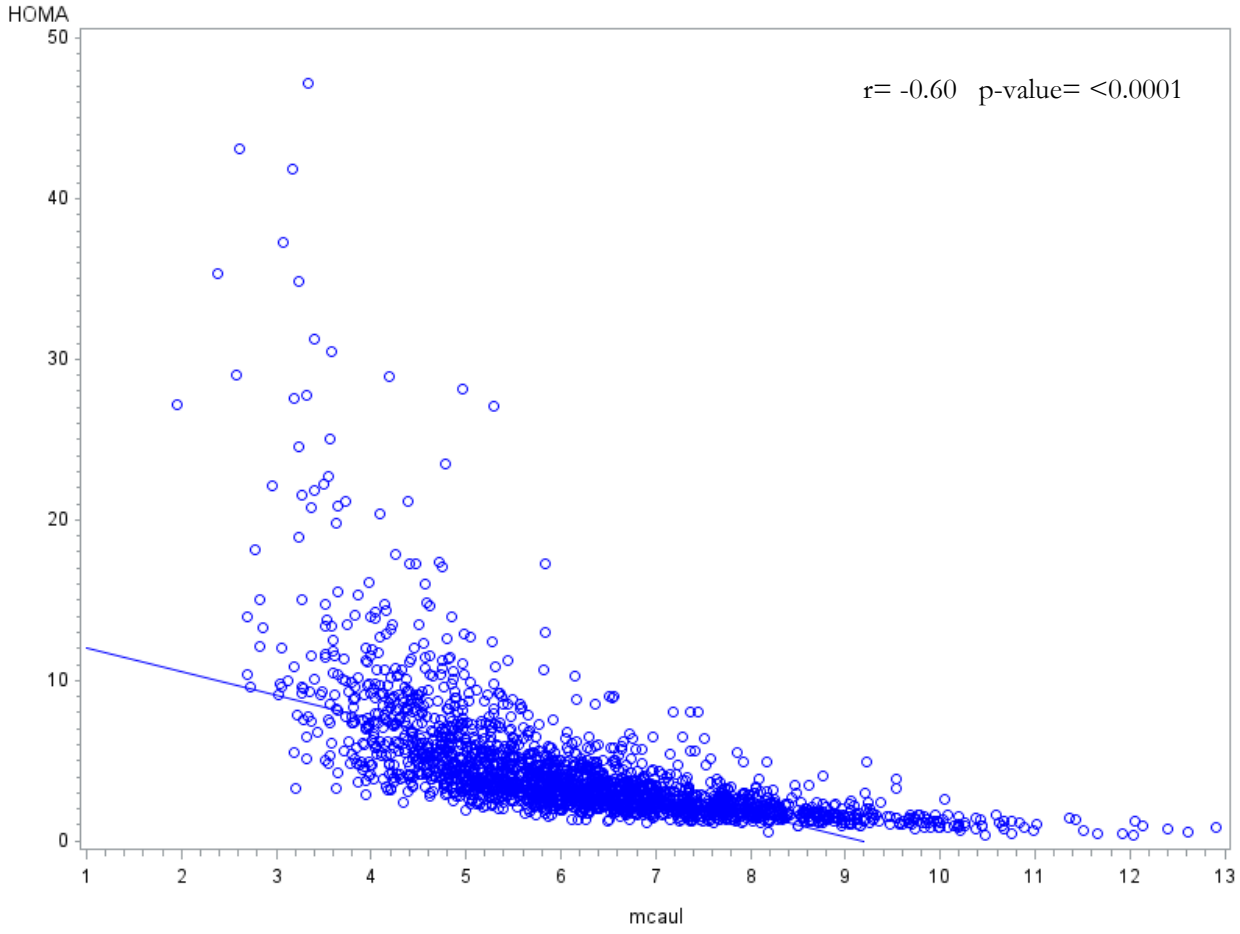


Table 2. Estimated adjusted associations (Odds Ratios [OR] and 95% confidence intervals [CI]) of Alzheimer's disease by insulin resistance status using the HOMA/McAuley/Combined indices: results of multivariate logistic regression analysis

Insulin Resistance (IR) Index	IR Status	No. of Subjects	No. of Alzheimer's Disease Cases	Unadjusted Odds Ratio (95% CI)	Model 1* Odds Ratio (95% CI)	Model 2** Odds Ratio (95% CI)
HOMA	Yes	1,477	41	0.82 (0.55-1.21)	0.89 (0.59-1.36)	0.89 (0.58-1.37)
	No	666	75	referent	referent	referent
McAuley	Yes	926	38	0.63 (0.42-0.93)	0.68 (0.45-1.02)	0.66 (0.43-1.01)
	No	1,217	78	referent	referent	referent
Combined	Yes	895	38	0.67 (0.45-0.99)	0.72 (0.47-1.08)	0.66 (0.43-1.01)
	No	1,248	78	referent	referent	referent

*Analyses were adjusted for age, BMI, alcohol, change in total cholesterol

**Analyses were adjusted for age, BMI, smoking, APOE ε4, alcohol, change in total cholesterol, prevalent hypertension at Exam 4, education, prevalent diabetes, prevalent CHD at Exam 4

Table 3. Estimated adjusted associations (Odds Ratios [OR] and 95% confidence intervals [CI]) of dementia by insulin resistance status using the HOMA/McAuley/Combined indices: results of multivariate logistic regression analysis

Insulin Resistance (IR) Index	IR Status	No. of Subjects	No. of Dementia Cases	Unadjusted Odds Ratio (95% CI)	Model 1* Odds Ratio (95% CI)	Model 2** Odds Ratio (95% CI)
HOMA	Yes	1,477	130	0.85 (0.62-1.16)	0.95 (0.69-1.323	0.87 (0.62-1.23)
	No	666	68	referent	referent	referent
McAuley	Yes	926	68	0.66 (0.49-0.90)	0.73 (0.53-1.00)	0.67 (0.48-0.93)
	No	1,217	130	referent	referent	referent
Combined	Yes	895	68	0.71 (0.52-0.96)	0.78 (0.56-1.07)	0.71 (0.51-0.99)
	No	1,248	130	referent	referent	referent

*Analyses were adjusted for age, BMI, alcohol, change in total cholesterol

**Analyses were adjusted for age, BMI, smoking, APOE ε4, alcohol, change in total cholesterol, prevalent hypertension at Exam 4, education, prevalent diabetes, prevalent CHD at Exam 4

Table 4. Estimated adjusted associations (Odds Ratios [OR] and 95% confidence intervals [CI]) of Alzheimer's disease by insulin resistance status using the Combined IR index: results of stratified multivariate logistic regression analysis by APOE ε4, hypertension, coronary heart disease, Type 2 Diabetes status

Stratified Variables		IR Status	No. of Cases	OR*
APOE ε4	Yes (n=387)	Yes (n=181)	11	0.64 (0.27-1.50)
		No (n=206)	17	referent
	No (n=1,756)	Yes (n=714)	27	0.71 (0.43-1.17)
		No (n=1,042)	61	referent
Hypertension	Yes (n=1,601)	Yes (n=729)	32	0.80 (0.50-1.30)
		No (n=872)	52	referent
	No (n=542)	Yes (n=166)	6	0.42 (0.16-1.11)
		No (n=376)	26	referent
CHD	Yes (n=411)	Yes (n=200)	8	1.20 (0.41-3.53)
		No (n=211)	8	referent
	No (n=1,732)	Yes (n=695)	30	0.63 (0.40-1.01)
		No (n=1,037)	70	referent
Type 2 Diabetes	Yes (n=596)	Yes (n=344)	18	0.92 (0.44-1.92)
		No (n=252)	17	referent
	No (n=1,547)	Yes (n=551)	20	0.63 (0.37-1.09)
		No (n=996)	61	referent

*Analyses were adjusted for age, BMI, smoking, alcohol, change in total cholesterol, prevalent hypertension at exam 4, education, prevalent diabetes, and prevalent CHD at exam 4

Table 5. Table 4. Estimated adjusted associations (Odds Ratios [OR] and 95% confidence intervals [CI]) of dementia by insulin resistance status using the Combined IR index: results of stratified multivariate logistic regression analysis by APOE ε4, hypertension, coronary heart disease, Type 2 Diabetes status

Stratified Variables		IR Status	No. of Cases	OR*
APOE ε4	Yes (n=387)	Yes (n=181)	20	0.80 (0.41-1.57)
		No (n=206)	26	referent
	No (n=1,756)	Yes (n=714)	48	0.67 (0.46-0.98)
		No (n=1,042)	104	referent
Hypertension	Yes (n=1,601)	Yes (n=729)	57	0.72 (0.50-1.04)
		No (n=872)	96	referent
	No (n=542)	Yes (n=166)	11	0.65 (0.30-1.42)
		No (n=376)	34	referent
CHD	Yes (n=411)	Yes (n=200)	17	0.72 (0.35-1.47)
		No (n=211)	23	referent
	No (n=1,732)	Yes (n=695)	51	0.70 (0.48-1.02)
		No (n=1,037)	107	referent
Type 2 Diabetes	Yes (n=596)	Yes (n=344)	35	0.93 (0.54-1.62)
		No (n=252)	30	referent
	No (n=1,547)	Yes (n=551)	33	0.62 (0.40-0.96)
		No (n=996)	100	referent

*Analyses were adjusted for age, BMI, smoking, APOE ε4, alcohol, change in total cholesterol, prevalent hypertension at Exam 4, education, prevalent diabetes, prevalent CHD at Exam 4

Chapter 3

Is there an Association between increased Fibrinogen and Insulin Resistance?

Introduction

The World Health Organization's 2014 global report on diabetes states that the prevalence of type 2 diabetes (diabetes) has risen from 108 million in 1980 to 422 million in 2014. While not the only risk factor for diabetes, insulin resistance (IR) is the most powerful predictor of diabetes.[100] IR has been shown to be closely tied to inflammatory processes.[101, 102]

Correlation between increased C-reactive protein levels, fibrinogen levels, IL-6 counts, and white cell counts have been found with incident diabetes.[101] The chronic inflammatory response to IR has been linked to many inflammatory cytokines, including TNF-α and IL-6.[103] One of the roles TNF-α plays is in the stimulation of hepatic acute phase proteins, such as fibrinogen.[31, 104] IL-6 is also associated with an increase of fibrinogen.[105] It is unclear whether fibrinogen plays a mechanistic part in IR, or serves purely as a marker of inflammation. Fibrinogen's main role is in the formation of blood clots, but also plays a role in the pro-inflammatory response in cardiovascular disease.[35, 106]

Findings from epidemiologic studies that have investigated a relationship between insulin resistance (IR) and fibrinogen are limited. Fibrinogen has been positively associated with IR[31, 32], but not yet investigated in Japanese populations. The aim of this study is to investigate whether fibrinogen are associated with IR in participants of the Honolulu Asia Aging Study.

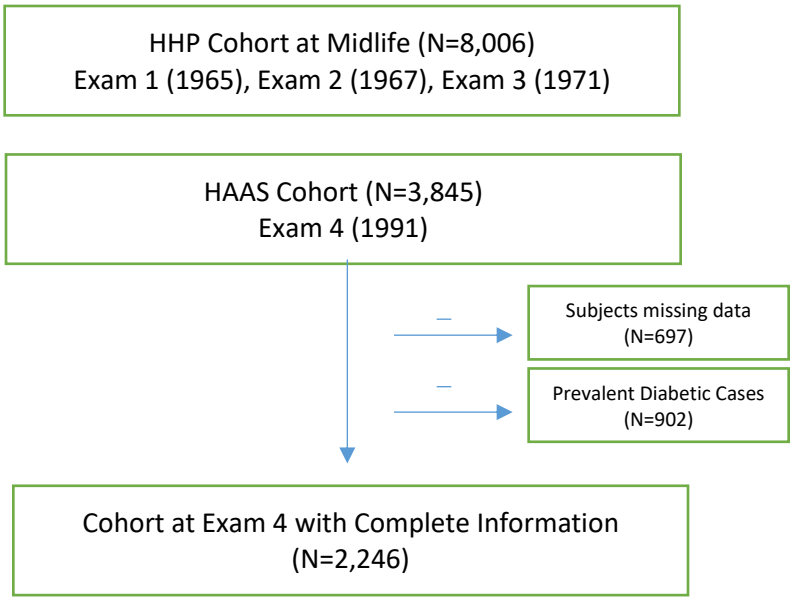
Methods

Study Population

Data were obtained from the Honolulu Heart Program (HHP), a longitudinal study established to study heart disease and stroke in a cohort of 8,006 Japanese-American men born between 1900 and 1919 and living in Oahu. Baseline data was taken in the first exam that began in 1965 and ended in 1968. Follow up examinations occurred during the years 1967-1970 (Exam 2) and 1971-1975 (Exam 3). The Honolulu Asia Aging Study (HAAS) was established in 1991-1993 (Exam 4) to study diseases relating to aging and

neurologic disorders. Blood samples and all other demographic and anthropomorphic data was ascertained from exam 4.

Figure 4. Selection process of the cohort for the fibrinogen-insulin resistance study



Assessment of Insulin Resistance

Insulin resistance was estimated using the Homeostatic Model Assessment (HOMA) and the McAuley index. HOMA-IR index was calculated as [fasting insulin (μU/mL) x fasting blood glucose (mg/dL) / 405].[55] Serum glucose was used instead of fasting blood glucose but should have a minimal effect on the outcome of HOMA.[56] McAuley index was calculated as exp[2.63- 0.28 x ln (fasting insulin (μU/ml))- 0.31 x ln (fasting triglycerides (mmol/l))].[57] The Japan Diabetes Society recommended HOMA values ≥2.5 as identifying IR and this cutoff has been used in previous Asian Studies; and a cut off of ≤5.8 based off the McAuley index was used.[57, 58] In addition to the McAuley index and HOMA-IR index, a third variable was created to indicate IR. Subjects who were considered IR by both the McAuley and HOMA indices were then considered IR in the Combined index. All other subjects in the Combined Index were considered non-IR.

Fibrinogen Measurements

Blood samples drawn from participants at exam 4 were sent to the Laboratory for Clinical Biochemistry Research at the University of Vermont, Colchester. Fibrinogen levels were determined on a BBL fibrometer and a semiautomated modification of the Clauss method defined the rate of clot formation. Quality control and calibration details have been described in prior publications.[107] Fibrinogen quartiles established as: ≤262 mg/mL Quartile 1, 263 to 293 mg/mL Quartile 2, 294 to 337 mg/mL Quartile ≥338 mg/mL Quartile 4.

Measure of C-reactive Protein

CRP measurements were assayed using nonfasting blood samples taken from all subjects of the HHP at exam 2. The assay was developed by Macy and Colleagues in the laboratory for Clinical Biochemistry Research, University of Vermont.[108] A 5.14% interassay coefficient of variation was used for this assay and the World Health Organization CRP reference standard was used.

Measure of Covariates and Modifiers

Later life age, waist circumference [109], smoking[68, 110, 111], hypertension[112-114], LDL cholesterol[115, 116], coronary heart disease (CHD)[70, 71, 107] and alcohol[117, 118] have all been identified as possible confounders in this study. Prevalent hypertension and prevalent CHD were also identified as possible effect modifiers.[119] Waist circumference was measured at exam 4. Prevalent hypertension was defined as systolic blood pressure ≥140 mm HG, diastolic blood pressure ≥90 mm HG or use of hypertensive medication. LDL cholesterol was calculated using the Friedwald Formula (LDL= total-HDL – TG/5) in men with triglyceride concentrations <400mg/dl.[120] Alcohol was measured in ounces per month consumed, then recoded into nondrinker, < 1 drink a day (up to 3 ounces per month), 1-2 drinks per day (3 to 30 ounces per month) and ≥3 drinks per day.[75] Smoking status was self-reported and categorized by never, past, or current smoker. CHD history was determined using surveillance data, in addition to questionnaire data and ECG at exam 4.[65]

Statistical Analysis

All statistical analysis was conducted with SAS software version 9 (SAS Institute, Cary, NC). Fibrinogen levels were divided into quartiles. Frequency statistics and comparisons of means and distributions were included in the univariate analysis using either t-tests for continuous variables or Pearson Chi Square test for categorical variables. Pearson correlation coefficients were calculated for: HOMA Index and McAuley Index, CRP and Fibrinogen, HOMA Index and Fibrinogen, and McAuley and Fibrinogen. For the analysis of IR, all subjects with diabetes were not included. Odds Ratios for IR by fibrinogen quartiles were estimated using logistic regression. In addition to the unadjusted model, two other models were included for the analysis. The first model includes identified confounders: age, WC, smoking status, alcohol consumption, and LDL cholesterol. Model 2 includes all variables in model 1 with the addition of possible effect modifiers and confounders: prevalent hypertension and prevalent coronary heart disease. In order to examine possible effect modification, I stratified by prevalent hypertensive status and prevalent coronary heart disease status.

Results

Baseline characteristics are shown in Table 6. Of the 2,246 subjects with complete information for fibrinogen and other variables at exam 4, 35% were IR according to the McAuley index, 59% were IR based on HOMA index, and 33% were IR according to the Combined index. Subjects divided by fibrinogen quartiles differed by age, LDL, smoking history, and HOMA index. Fibrinogen levels ranged from 164mg/L to 688 mg/L, with a median of 293mg/L. Figure 5 shows the correlation between HOMA index and McAuley index was significant ($r=-0.69$, p-value <0.0001). No significant correlation between midlife CRP and fibrinogen was found (Figure 6). Correlations between HOMA index/McAuley index with fibrinogen were weakly correlated. (Figures 7 and 8).

As shown in Tables 7 through 9, subjects in the third quartile of fibrinogen had a significantly higher odds of IR compared to the first quartile based on the McAuley and Combined indices, whereas subjects had higher odds of IR if in the fourth quartile using the HOMA index, compared to the first quartile. After

adjustment, the odds remained significant and even increased in magnitude. The addition of CHD or hypertension in the model did not affect the significant relationship between fibrinogen and IR. A significant p for trend was found in the final adjusted models using the McAuley and Combined indices.

Using the Combined index, an increase in waist circumference by a cm (OR 1.11 95% CI 1.10 - 1.13), prevalent hypertension (OR 1.67 95% CI 1.33 - 2.10), prevalent CHD (OR 1.29, 95% CI 1.01-1.66) and currently smoking (OR 1.60 95% CI 1.10 – 2.36) resulted in increased odds of IR. Additionally, subjects who were older (OR 0.97 95% CI 0.95 - 0.98) or subjects who drank three or more drinks a day (OR 0.69 95% CI 0.52 - 0.96) had a decreased odds of IR in the same model. The use of the HOMA index confirmed increased waist circumference (OR 1.12 95% CI 1.11 - 1.14) and prevalent hypertension (OR 1.70 95% CI 1.38 - 2.09) to increase odds of IR. Again, increased age (OR 0.97 95% CI0.95 - 0.99) and consumption of three or more alcoholic drinks (OR 0.58 95% CI 0.44 - 0.76) resulted in decreased odds of IR. Results from the McAuley index reflect those found in the HOMA index.

After stratification by hypertensive status, elevated fibrinogen was not associated with increased odds of IR using the Combined Index in non-hypertensive subjects (Table 10). However, amongst hypertensive subjects, fibrinogen was positively associated with IR. Comparing the fourth quartiles between hypertensive and normal-tensive subjects, the OR is quite different. Additionally, stratification by CHD status revealed subjects without CHD had much higher odds of IR compared to subjects with CHD, however the results from the CHD strata are not significant (Table 11).

Discussion

The data from this large sample of Japanese American men show subjects with elevated fibrinogen have increased odds of prevalent IR. The odds of IR using the Combined and McAuley indices increased significantly with subjects in the third quartile and the odds of IR increased in subjects in the fourth quartile using the HOMA index. The association was independent of potential confounders.

A few other studies have shown associations between fibrinogen and IR[31, 32]. In studies of non-Japanese cohorts, fibrinogen was found to be associated with IR. However, only Chen et al. utilized the HOMA index. Previous studies examining fibrinogen and IR in Japanese populations have mixed results.

One study found that young, non-diabetic Japanese adults with elevated fibrinogen levels had a 28% increased odds of IR, using HOMA[121]. However, results from the Jichi Medical School Cohort Study did not find any significant differences of fibrinogen levels between fasting insulin tertiles.[122] A study of older, non-diabetic, British men showed a weak relationship between HOMA and fibrinogen.[123]

Higher levels of TNF-α, interleukin-6 (IL-6), and interleukin-8 (IL-8), all part of the pro-inflammatory process, have been found in diabetic and IR patients.[124] The production of fibrinogen has shown to be increased significantly by IL-6, and IL-6 was found to be associated with fibrinogen.[123, 125] Impaired insulin signaling is one of the hallmarks of IR, and has been tied to IL-6.[126, 127] Fibrinogen induction of increased IL-8 was proven by Qi and Kreutzer.[128] Recent studies have shown that TNF-α does not increase fibrinogen production.[129] Nonetheless, these biologic findings support an association between fibrinogen and IR.

The lack in correlation between later life fibrinogen and midlife CRP supports the thought that while CRP levels have been correlated up to five years apart, using CRP measurements from 20 years prior would not accurately account for inflammatory changes subjects would have experienced. Any further analysis looking at the relationship between CRP, as a marker of midlife inflammation, and later life IR would not be accurate.

The relationship between IR and hypertension and CHD has led to the clinical definition of the metabolic syndrome.[130] In the atherogenesis, insulin resistant mononuclear cells adhere to the endothelium with greater affinity than non-insulin resistant cells.[131] Multiple studies have shown fibrinogen to be associated with CHD and hypertension. Fibrinogen levels are related to left ventricular mass in hypertensive patients and fibrinogen was found in hypertensive-damaged organs.[132, 133] Ridker et al. found fibrinogen predicted peripheral arterial disease and Kazmi and Lwaleed found fibrinogen levels higher in patients with coronary artery disease versus angiographically normal subjects.[134, 135] A study of Japanese subjects identified fibrinogen as an independent risk factor of coronary atherosclerosis. [136] Among subjects in the same HAAS cohort, fibrinogen was associated with CHD.[107] The previous literature supports our findings

where elevated fibrinogen was associated with IR in subjects who were hypertensive or not suffering from CHD.

Cross-sectional studies have revealed that moderate alcohol consumption can result in anti-inflammatory effects and lower CRP levels.[137, 138] This cohort showed a decrease odds of IR in subjects who consumed three or more alcoholic drinks a day. Our study is in agreement with prior studies that showed a positive relationship between smoking and IR[139, 140] in which former smokers had a 20% increased odds of IR while the odds for IR in current smokers was almost double, compared to non-smokers.

Our findings add to the growing literature examining the usefulness of fibrinogen as a marker of inflammation with a possible association with IR. Some important strengths of this study exist. To our knowledge, this is the first study that investigated the relationship of fibrinogen and IR in an older, as well as Japanese, population, as well as the use of the McAuley index, to supplement the more widely used HOMA to determine IR status. To the best of our knowledge, this is the first study using the McAuley index to examine the relationship between CRP and IR in a Japanese population. The McAuley index is the only IR index utilizing triglyceride levels, and is best suited for epidemiological studies.[58] Also, CRP levels and insulin tend to be skewed in Japanese populations. Fibrinogen was normally distributed in this population. The restriction of the HAAS to Japanese men of the same age reduces confounding by age, ethnicity, sex-related factors and genetics.

Nevertheless, we are aware of the limitations of this study. Due to lack of information at exam 2, we were unable to accurately define IR status at exam 2. As a result, we were only able to look at the relationship between fibrinogen and odds of prevalent IR. The restrictions of the HAAS also serve as limitations to the generalizability to the study. The cross-sectional nature of this study limits our ability to draw causal inferences between the relationship between fibrinogen and CRP with IR. While findings show plausible mechanistic links between fibrinogen and CRP with IR, further studies looking at multiple measurements of those inflammatory markers are needed to truly see if fibrinogen are causally related to IR, instead of just markers of the inflammatory process created by IR. Due to recruitment and identification of participants using Census data and Selective Service registration records, selection bias is a possibility.

In conclusion, I observed that higher fibrinogen levels did result in increased odds of prevalent IR. While shorter prospective studies have shown an association, further research focusing on prolonged follow up periods would help clarify this relationship.

Table 6. Selected characteristics of Participants by Fibrinogen quartile: The Honolulu-Asia Aging Study

Variables	Fibrinogen Quartile 1 (≤262mg/L)	Fibrinogen Quartile 2 (263-293mg/L)	Fibrinogen Quartile 3 (294-337mg/L)	Fibrinogen Quartile 4 (≥338mg/L)	P-Value
n=2,246	564	568	555	559	
Age (years)	77.4 ± 4.2	77.9 ± 4.4	78.1 ± 4.4	77.9 ± 4.5	0.06
Waist Circumference (cm)	85.2 ± 8.4	84.9 ± 8.1	85.6 ± 8.8	85.8 ± 9.1	0.36
LDL Cholesterol (mg/dL)	102.8 ± 27.4	110.0 ± 29.8	114.1 ± 30.8	117.8 ± 32.1	<0.001
Prevalent CHD					
Yes	85 (3.78%)	89 (3.96%)	94 (4.19%)	110 (7.90%)	0.17
No	479 (21.33%)	479 (21.33%)	461 (20.53%)	449 (19.99%)	
Prevalent Hypertension (Y/N)					
Yes	412 (18.34%)	398 (17.72%)	398 (17.72%)	415 (18.48%)	0.44
No	152 (6.77%)	170 (7.57%)	157 (3.99%)	144 (6.41%)	
Smoking					
Never	221 (9.84%)	218 (9.71%)	218 (9.71%)	177 (7.88%)	<0.001
Past	301 (13.40%)	323 (14.38%)	293 (13.05%)	319 (14.20%)	
Current	42 (1.87%)	27 (1.20%)	44 (1.96%)	63 (2.80%)	
Alcohol Exam 2					
Non drinker	241 (10.73%)	228 (10.15%)	252 (11.22%)	219 (9.75%)	0.08
<1 a day	48 (2.14%)	49 (2.18%)	48 (2.14%)	38 (1.69%)	
1 to 2 a day	165 (7.35%)	199 (8.86%)	169 (7.52%)	183 (8.15%)	
3 or more	110 (4.90%)	92 (4.10%)	86 (3.83%)	119 (5.30%)	
HOMA (Insulin Resistant)					
Yes	324 (14.43%)	308 (13.71%)	337 (15.00%)	354 (15.76%)	0.01
No	240 (10.69%)	260 (11.58%)	218 (9.71%)	205 (9.13%)	

Table 6. (Continued) Selected characteristics of Participants by Fibrinogen quartile: The Honolulu-Asia Aging Study

McAuley (Insulin Resistant)					
Yes	178 (7.93%)	186 (8.28%)	213 (9.48%)	202 (8.99%)	0.06
No	386 (17.19%)	382 (17.01%)	342 (15.23%)	357 (15.89%)	
Combined (Insulin Resistant)					
Yes	166 (7.39%)	180 (8.01%)	200 (8.90%)	195 (8.68%)	0.075
No	398 (17.72%)	388 (1728%)	355 (15.81%)	364 (16.21%)	

Figure 5. Correlation between HOMA index and the McAuley Index

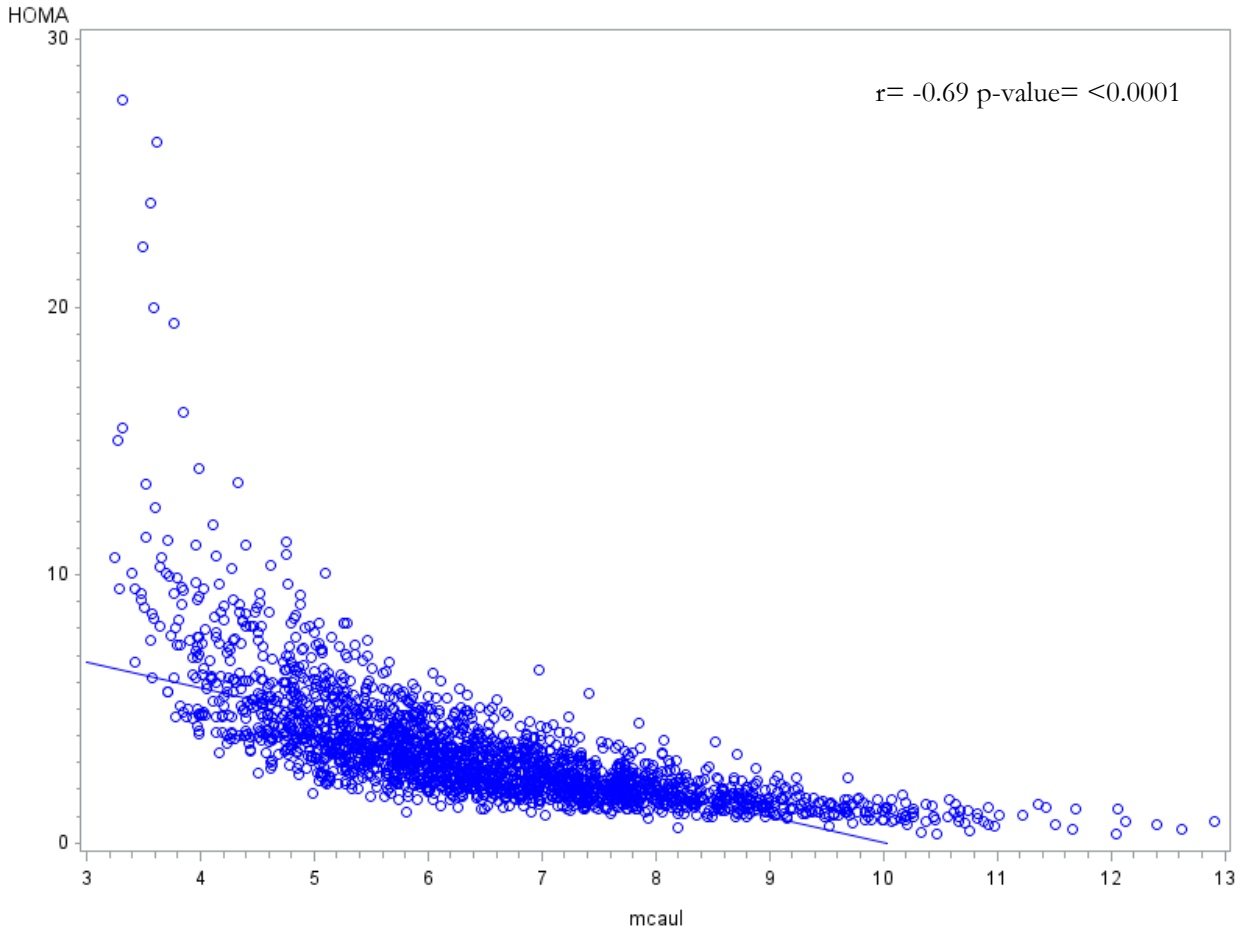


Figure 6. Correlation between midlife C-Reactive protein and later life Fibrinogen

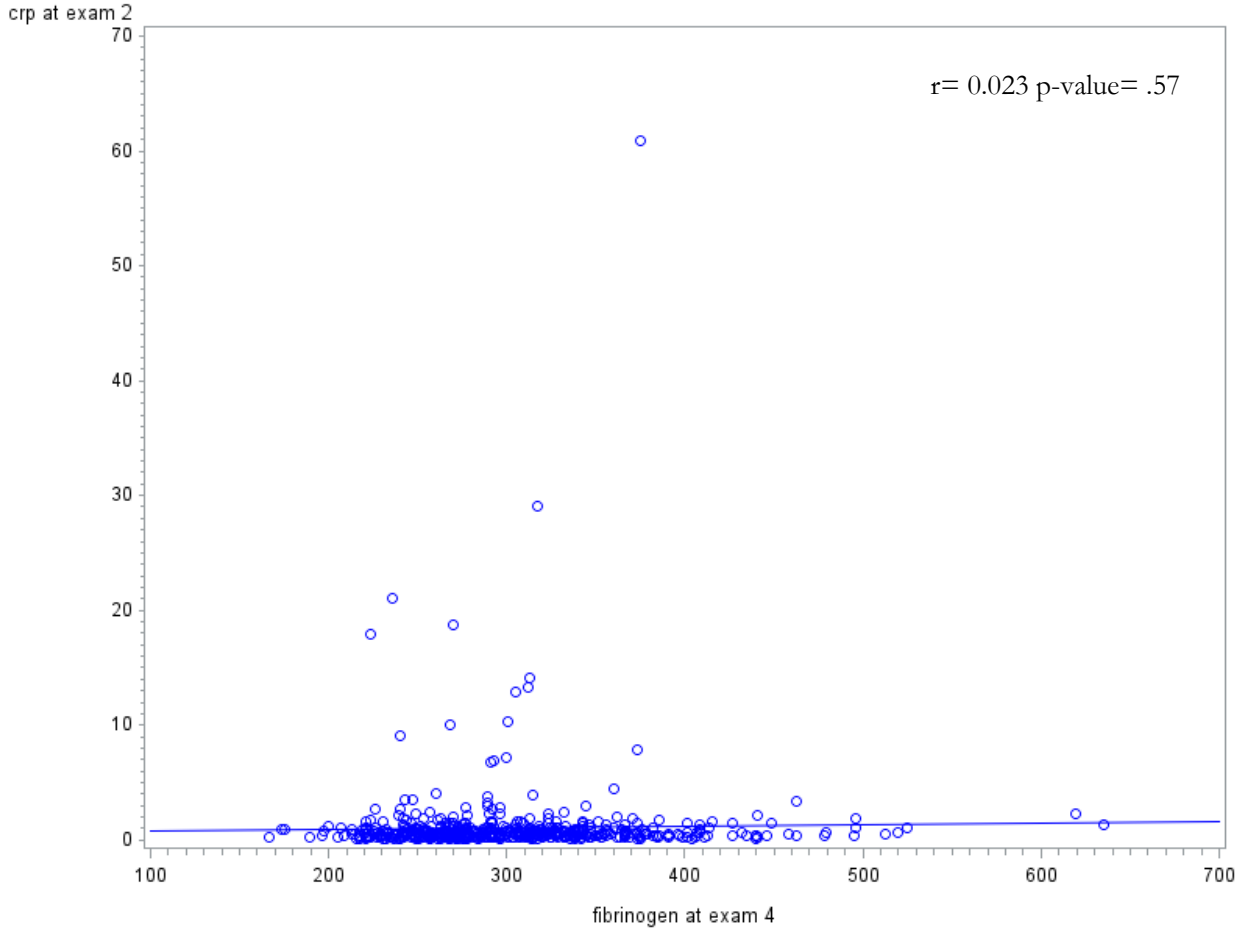


Figure 7. Correlation between later life Fibrinogen and HOMA index

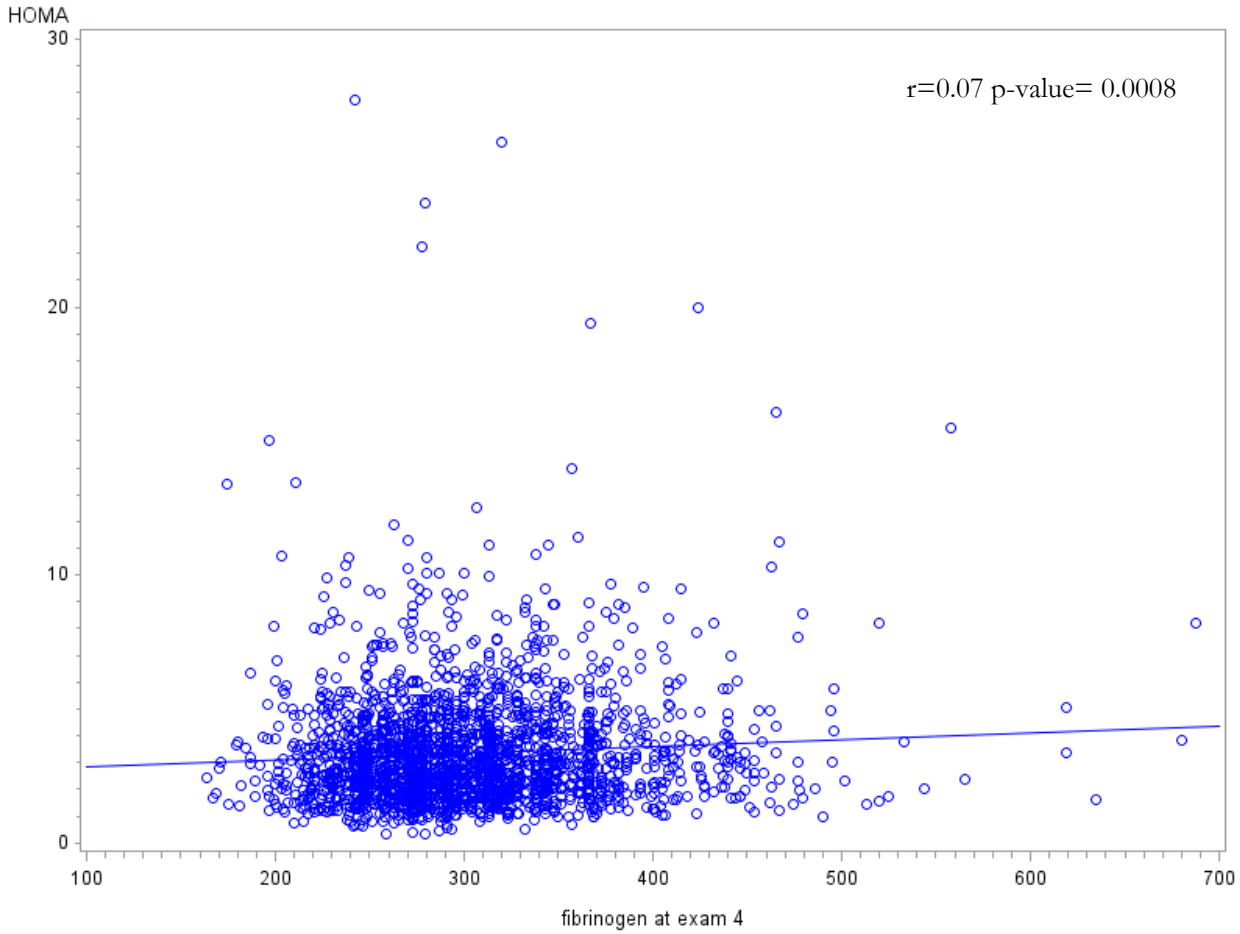


Figure 8. Correlation between later life Fibrinogen and McAuley index

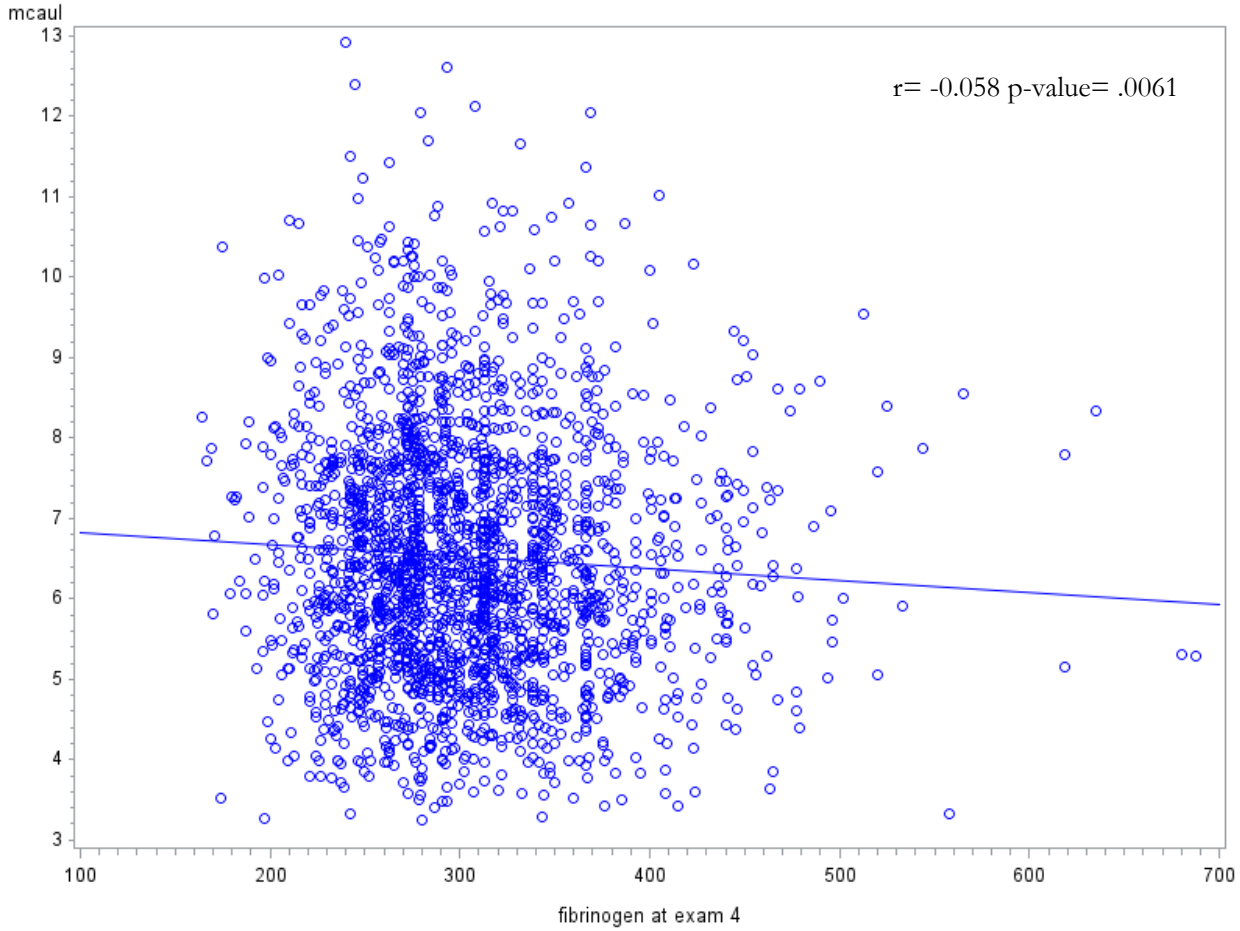


Table 7. Estimated adjusted associations (Odds Ratios [OR] and 95% confidence intervals [CI]) of insulin resistance by fibrinogen quartiles using the HOMA IR index: results of multivariate logistic regression analysis

HOMA	Number of IR Cases	Unadjusted	Adjusted for Confounders*	Adjusted for confounders and modifiers**
Fibrinogen Quartile 1 (≤262) N=564	324	referent	referent	referent
Fibrinogen Quartile 2 (263 to 293) N=568	308	0.88 (0.69−1.11)	0.90 (0.69−1.16)	0.91 (0.70−1.18)
Fibrinogen Quartile 3 (294 to 337) N=555	337	1.15 (0.90−1.45)	1.15 (0.88−1.50)	1.16 (0.89−1.52)
Fibrinogen Quartile 4 (≥338) N=559	354	1.28 (1.01−1.63)	1.33 (1.02−1.74)	1.32 (1.00−1.70)
P for trend				p= 0.01

*Analyses were adjusted for age, waist circumference, smoking alcohol, LDL cholesterol

**Analyses were adjusted for age, waist circumference, smoking, alcohol, LDL cholesterol, prevalent hypertension and coronary heart disease

Table 8. Estimated adjusted associations (Odds Ratios [OR] and 95% confidence intervals [CI]) of insulin resistance by fibrinogen quartiles using the McAuley IR index: results of multivariate logistic regression analysis

McAuley	Number of IR Cases	Unadjusted	Adjusted for confounders*	Adjusted for confounders and modifiers**
Fibrinogen Quartile 1 (≤262) N=564	178	referent	referent	referent
Fibrinogen Quartile 2 (263 to 293) N=568	186	1.06 (0.82−1.36)	1.16 (0.89−1.52)	1.19 (0.91−1.56)
Fibrinogen Quartile 3 (294 to 337) N=555	213	1.35 (1.06−1.73)	1.43 (1.09−1.88)	1.46 (1.10−1.92)
Fibrinogen Quartile 4 (≥338) N=559	202	1.23 (0.96−1.57)	1.26 (0.96−1.66)	1.27 (0.96−1.67)
P for Trend				p= 0.06

*Analyses were adjusted for age, waist circumference, smoking alcohol, LDL cholesterol

**Analyses were adjusted for age, waist circumference, smoking, alcohol, LDL cholesterol, prevalent hypertension and coronary heart disease

Table 9. Estimated adjusted associations (Odds Ratios [OR] and 95% confidence intervals [CI] of insulin resistance by fibrinogen quartiles using the Combined IR index: results of multivariate logistic regression analysis

Combined Index	Number of IR Cases	Unadjusted	Adjusted for Confounders*	Adjusted for confounders and modifiers**
Fibrinogen Quartile 1 (≤262) N=564	166	referent	referent	referent
Fibrinogen Quartile 2 (263 to 293) N=568	180	1.11 (0.86-1.43)	1.22 (0.93-1.62)	1.26 (0.96-1.67)
Fibrinogen Quartile 3 (294 to 337) N=555	200	1.35 (1.05-1.74)	1.40 (1.07-1.85)	1.43 (1.08-1.89)
Fibrinogen Quartile 4 (≥338) N=559	195	1.28 (1.00-1.65)	1.31 (0.99-1.73)	1.31 (0.99-1.74)
P for trend				p=0.041

*Analyses were adjusted for age, waist circumference, smoking alcohol, LDL cholesterol

**Analyses were adjusted for age, waist circumference, smoking, alcohol, LDL cholesterol, prevalent hypertension and coronary heart disease

Table 10. Estimated adjusted associations (Odds Ratios [OR] and 95% confidence intervals [CI]) of insulin resistance by fibrinogen quartiles using the Combined IR index, stratified by hypertension: results of multivariate logistic regression analysis

Stratification by Hypertension*	Cases of IR	Fibrinogen Quartile 1 (≤262)	Fibrinogen Quartile 2 (263 to 293)	Fibrinogen Quartile 3 (294 to 337)	Fibrinogen Quartile 4 (≥338)
McAuley Index					
Hypertensive (N=1,623)	613	referent	1.18 (0.89−1.61)	1.50 (1.10−2.06)	1.36 (1.00−1.87)
Not hypertensive (N=623)	166	referent	1.20 (0.70−2.10)	1.30 (0.76−2.40)	1.00 (0.53−1.71)
HOMA Index					
Hypertensive (N=1,623)	1,106	referent	1.01 (0.74−1.37)	1.30 (0.92−1.72)	1.50 (1.05−2.00)
Not hypertensive (N=623)	307	referent	0.69 (0.42−1.10)	0.95 (0.57−1.60)	0.99 (0.59−1.70)
Combined Index					
Hypertensive (N=1,623)	585	referent	1.25 (0.91-1.72)	1.44 (1.05-1.98)	1.39 (1.01-1.91)
Not hypertensive (N=623)	156	referent	1.29 (0.73-2.29)	1.44 (0.80-2.57)	1.06 (0.57-1.95)

*Analyses were adjusted for age, waist circumference, smoking, alcohol, LDL cholesterol, coronary heart disease

Table 11. Estimated adjusted associations (Odds Ratios [OR] and 95% confidence intervals [CI]) of insulin resistance by fibrinogen quartiles using the Combined IR indices, stratified by coronary heart disease: results of multivariate logistic regression analysis

Stratification by CHD*	Cases of IR	Fibrinogen Quartile 1 (≤262)	Fibrinogen Quartile 2 (263 to 293)	Fibrinogen Quartile 3 (294 to 337)	Fibrinogen Quartile 4 (≥338)
McAuley Index					
CHD (N=378)	152	referent	0.56 (0.28-1.09)	1.04 (0.53-2.03)	0.62 (0.32-1.19)
No CHD (N=1,868)	627	referent	1.37 (1.01-1.84)	1.56 (1.15-2.11)	1.84 (1.09-2.02)
HOMA Index					
CHD (N=378)	249	referent	0.56 (0.27-1.14)	0.68 (0.33-1.43)	0.90 (0.44-1.85)
No CHD (N=1,868)	1,074	referent	0.97 (0.73-1.28)	1.27 (0.95-1.69)	1.39 (1.03-1.87)
Combined Index					
CHD (N=378)	150	referent	0.63 (0.32-1.24)	1.18 (0.60-2.31)	0.71 (0.37-1.37)
No CHD (N=1,868)	591	referent	1.43 (1.05-1.95)	1.49 (1.09-2.03)	1.51 (1.11-2.07)

* Analyses were adjusted for age, waist circumference, smoking, alcohol, LDL cholesterol, coronary heart disease

Chapter 4

Is there an Association between increased Fibrinogen and Type 2 Diabetes?

Introduction

Understanding the body's inflammatory response to diseases is a key part of figuring out disease pathology. While it is clear that inflammation occurs in patients with type 2 diabetes (diabetes), the exact mechanisms and the causal relationship of inflammation with type 2 diabetes is still unclear.[141] Of the studied inflammatory markers, fibrinogen has been used repeatedly in the study of type 2 diabetes. Fibrinogen is an acute phase protein and subjects with type 2 diabetes have displayed elevated levels of fibrinogen.[33, 34] Fibrinogen levels have also been shown to be reduced by insulin infusions.[142]

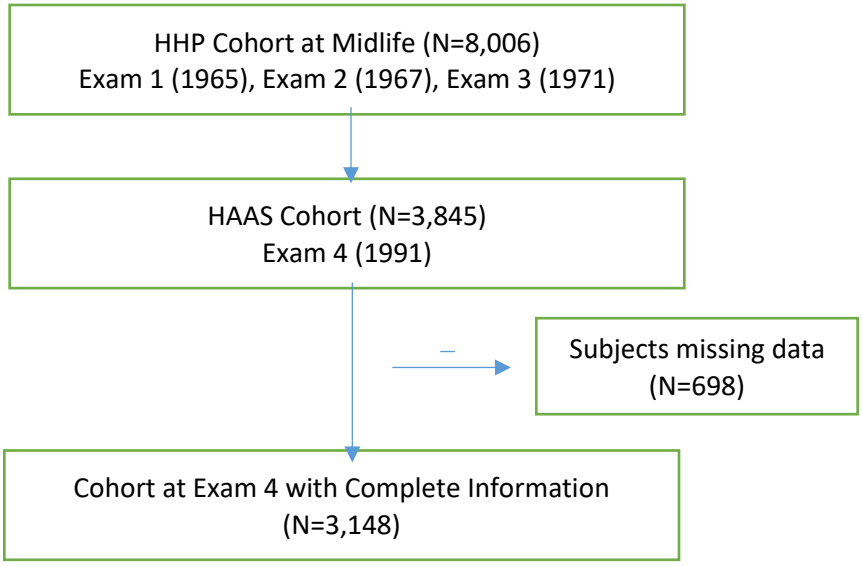
Diabetes is a chronic disease that is affecting people globally, including the United States and Asia.[143] Diabetes is characterized by abnormal beta-cell function in the pancreas, caused by increased insulin resistance (IR)[100, 144] Other contributing factors for the rise in the diabetic epidemic is the association of diabetes with obesity and cardiovascular risk factors.[145] Epidemiologic studies have shown an association with fibrinogen and diabetes.[104, 146]

The purpose of this study is to examine the relationship between fibrinogen and diabetes in subjects of the Honolulu Asia Aging study.

Methods

The Honolulu Asia Aging Study (HAAS) was founded in 1991 as a continuation of the Honolulu Heart Program(HHP), a longitudinal study established to study heart disease and stroke in a cohort of 8,006 Japanese-American men born between 1900 and 1919 living in Oahu. The focus of the HAAS shifted from cardiovascular disease to aging and neurologic disorders. This is analysis is based on blood samples and all other anthropomorphic and background data obtained at Exam 4, the first exam of the HAAS.

Figure 9. Selection process of the cohort for the fibrinogen-type 2 diabetes study



Assessment of Diabetes

Diabetes was assessed using self-report of doctor's diagnosis, use of insulin intake or oral hypoglycemic medications. Additionally, subjects who were not diabetic were asked to complete a fasting and two hour post load glucose tolerance test. Based on the definition according to the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, subjects who had a fasting glucose of >126mg/dl or two-hour glucose level of >200mg/dl were classified as diabetic.[147]

Assessment of Insulin Resistance

Insulin resistance was estimated using the Homeostatic Model Assessment (HOMA) and the McAuley index. HOMA-IR index was calculated as [fasting insulin (μU/mL) x fasting blood glucose (mg/dL) / 405].[55] Serum glucose was used instead of fasting blood glucose but should have a minimal effect on the outcome of HOMA.[56] McAuley index was calculated as exp[2.63- 0.28 x ln (fasting insulin (μU/ml))- 0.31 x ln (fasting triglycerides (mmol/l))].[57] The Japan Diabetes Society recommended HOMA values ≥2.5 as identifying IR and this cutoff has been used in previous Asian Studies; and a cut off of ≤5.8 based off the McAuley index was used.[57, 58] In addition to the McAuley index and HOMA-IR index, a third variable was created to indicate IR. Subjects who were considered IR by both the McAuley and

HOMA indices were then considered IR in the Combined index. All other subjects in the Combined Index were considered non-IR.

Fibrinogen Measurements

Blood samples drawn from participants at Exam 4 were sent to the Laboratory for Clinical Biochemistry Research at the University of Vermont, Colchester. Fibrinogen levels were determined on a BBL fibrometer and a semiautomated modification of the Clauss method defined the rate of clot formation. Quality control and calibration details have been described in prior publications.[107] Fibrinogen quartiles were established as: ≤263 mg/mL Quartile 1, 264 to 296 mg/mL Quartile 2, 297 to 338 mg/mL Quartile 3, and ≥339 mg/mL Quartile 4.

Measure of C-reactive Protein

CRP measurements were assayed using nonfasting blood samples taken from all subjects of the HHP at exam 2. The assay was developed by Macy and Colleagues in the laboratory for Clinical Biochemistry Research, University of Vermont.[108] A 5.14% interassay coefficient of variation was used for this assay and the World Health Organization CRP reference standard was used.

Measures of Covariates and Modifiers

Possible confounders identified in this study include age at Exam 4, waist circumference [66, 109], hypertension [112, 148], smoking[111, 149], LDL cholesterol[150, 151], alcohol[117, 152] and coronary heart disease (CHD)[153-155]. Prevalent hypertension, prevalent CHD, and prevalent IR were also identified as possible effect modifiers. Waist circumference was measured at Exam 4. Prevalent hypertension was defined as systolic blood pressure ≥140 mm HG, diastolic blood pressure ≥90 mm HG or use of hypertensive medication[74]. Alcohol was measured in ounces per month consumed, then recoded into non drinker, < 1 drink a day (up to 3 ounces per month), 1-2 drinks per day (3 to 30 ounces per month) and ≥3 drinks per day.[75] Smoking status was self-reported and categorized by never, past, or current smoker. CHD history

was determined using surveillance data, in addition to questionnaire data and ECG at Exam 4.[65] LDL cholesterol was calculated using the Friedwald Formula (LDL= total- HDL – TG/5) in men with triglyceride concentrations <400mg/dl.[120]

Statistical Analysis

Statistical analysis was conducted with SAS software version 9.4 (SAS Institute, Cary, NC). Fibrinogen levels were divided into quartiles. Frequency statistics and comparisons of means and distributions were included in the univariate analysis using either t-tests for continuous variables or Pearson Chi Square test for categorical variables. Pearson correlation coefficients were calculated for: HOMA Index and McAuley Index, CRP and Fibrinogen, HOMA Index and Fibrinogen, and McAuley and Fibrinogen. Odds Ratios for diabetes by fibrinogen quartiles were estimated using logistic regression. In addition to the unadjusted model, two other models were included for the analysis. The first model included these possible confounders: age, waist circumference, smoking status, alcohol consumption, and LDL cholesterol. The second model includes variables from model one, and also includes prevalent hypertension, prevalent CHD, and prevalent IR as additional possible confounders. P for trend analysis was conducted on the final adjusted models. Stratified analysis was utilized to test for possible effect modification by prevalent CHD, prevalent hypertension, and prevalent IR.

Results

Of the 3,148 subjects at Exam 4 with complete information, 28.6% of them were classified as diabetic. Subjects who were diabetic differed significantly compared to non-diabetics by waist circumference, prevalent hypertension status, and prevalent CHD status (Table 12). Across quartiles of fibrinogen, significant differences were found in smoking status, LDL cholesterol, CHD status, IR status (HOMA index, McAuley index, Combined index), and diabetic status. Fibrinogen levels ranged from 123mg/L to 688mg/L, with a median of 296mg/L. A modest correlation (r=-0.46, p-value=<0.0001) between HOMA index and McAuley index was found (Figure 10). Correlation between midlife CRP and later life fibrinogen was not significant and minimal (Figure 11). Correlations between fibrinogen and HOMA index/McAuley index were both small, but significant (Figures 12 and 13).

The initial model showed significantly higher odds of prevalent diabetes for subjects in the third and fourth quartiles compared to the referent quartile (Table 13). The odds of diabetes for subjects in the fourth quartile of fibrinogen before adjusting for IR (OR 1.53 95% CI 1.22 - 1.91) were higher compared to the odds of diabetes after adding IR into the model (OR 1.40 95% CI 1.11 - 1.76). Significant p for trend was identified in the final model using all three IR indices.

Subjects who had higher waist circumference had a small, but significantly increased odds of diabetes using the McAuley index (OR 1.02, 95% CI 1.01 - 1.03). Subjects who were hypertensive at Exam 4 tended to be diabetic more than non-hypertensive subjects regardless of use of the McAuley index, HOMA index or Combined index, respectively (OR 1.31, 95% CI 1.08 - 1.60/ OR 1.28 95% CI 1.05 - 1.60/ OR 1.30 95% CI 1.07 - 1.58) . This association was also observed in subjects with CHD compared to non-CHD subjects, again using McAuley index, HOMA index, Combined index, respectively (OR 1.75 95% CI 1.45 - 2.12/ OR 1.67 95% CI 1.38 - 2.03, OR 1.30 95% CI 1.44 - 2.10). Subjects who were IR based on the Combined index had an increased odds of diabetes (OR 2.17 95% CI 1.82 - 2.58). Using the McAuley index or HOMA index, the OR for diabetes in IR subjects was 2.03 (95% CI 1.71 - 2.41) and 3.45 (95% CI 2.79 – 4.27) respectively.

We then stratified by IR, hypertensive, and CHD statuses. Stratification by IR yielded mixed results, depending on which IR index was used (Table 14). Using either the McAuley index or Combined index, subjects who were IR and in the second and fourth quartiles of fibrinogen had higher odds of diabetes than their non-IR counterparts. However, using the HOMA index, IR subjects in the fourth quartile of fibrinogen had lower odds of diabetes compared to non-IR subjects, however results were not statistically significant. Hypertensive patients had higher odds of prevalent diabetes compared to non-hypertensive patients, however the results amongst the non-hypertensive patients were not statistically significant (Table 15). However, subjects who were hypertensive were at increased odds of diabetes if they were in the third or fourth quartile of fibrinogen, and their odds were greater than the adjusted model. Stratification by CHD status revealed no difference in prevalent diabetes by fibrinogen quartile amongst subjects with CHD (Table 16).

Discussion

This study adds to the sparse literature regarding the association between fibrinogen and diabetes. Our study found subjects with elevated fibrinogen levels had higher odds of prevalent diabetes, especially among subjects who were IR or hypertensive. Establishing associations between fibrinogen, diabetes, IR and hypertension is the first step to understanding the causal mechanisms.

Our findings confirm results from prior studies that also looked at the relationship between fibrinogen and diabetes.[104, 146, 156] The Multi-Ethnic Study of Atherosclerosis (MESA) found fibrinogen predicted increased risk of diabetes with subjects in the highest quartile of fibrinogen (HR 1.5, 95% CI 1.1, 2.2). However, the relationship no longer existed once the model was adjusted for HOMA and BMI. A similar situation occurred in Insulin Resistance Atherosclerosis Study, where the association between incident diabetes and fibrinogen was significantly attenuated after adjusting for body fat.[104]

This is the first study to examine fibrinogen and diabetes in Japanese men, and also to look at the interaction between fibrinogen and IR in relation to diabetes. In contrast to the MESA study, subjects in the highest quartile of fibrinogen still had significant odds of diabetes, after adjustment of IR and waist circumference. The use of waist circumference has proven to be a better indicator of visceral fat then BMI and waist to hip ratio, especially in Japanese populations, who tend to have lower BMI than other ethnicities.[66]

Our investigation into IR as an effect modifier proved inconclusive. Stratification of IR using the McAuley index and Combined index showed a higher odds of diabetes for IR subjects in the second and fourth fibrinogen quartiles. However, that relationship was reversed in the third quartile. When using HOMA to define IR status, those that were IR across all quartiles of fibrinogen had higher odds of diabetes, compared to non-resistant subjects. Yet, the difference in OR estimates comparing the stratified models and non-stratified models is minimal. A recent study by Grossman et al. found fibrinogen concentrations rose in subjects with normal hemoglobin a1c levels to subjects with prediabetes, and fibrinogen concentrations only weakly increased from prediabetic patients to diabetic patients.[157]

Stratification by hypertension in our subjects showed hypertension to be a possible effect modifier. The odds of diabetes in normal-hypertensive subjects by fibrinogen quartile are lower compared to hypertensive subjects. The prevalence of hypertension in American type 2 diabetics occurs between 50%-80% and a prospective study in the United States found the risk of diabetes in hypertensive patients was 2.5 times compared to normal-tensive patients.[158, 159] In subjects of the Osaka Health Survey, hypertensive patients had an adjusted risk ratio for diabetes of 1.39(CI 1.14 - 1.69) compared to normal-tensive patients.[160]

While Fibrinogen's role in platelet aggregation is vital towards the wound healing processes, the formation of fibrin via fibrinogen is also a key player in accumulation of fatty deposits and scar tissues, which lead to atherosclerosis.[157, 161] Subjects with CHD had higher odds of diabetes, however, I did not observe any major differences between the patients based on CHD status, across fibrinogen quartiles.

Low grade inflammation is found in both hypertension and diabetes, and shares common pathways that includes oxidative stress, adipokines, and IR.[162] IL-6 is a pro-inflammatory cytokine that has been associated with both IR and diabetes.[127, 163] IL-6 was previously thought to be neither necessary nor sufficient in the development of type 1 and type 2 diabetes. However, recent findings suggest IL-6 negatively affects insulin signaling processes, which contributes to IR.[36, 126] IL-6 can increase the production of fibrinogen, and has been associated with higher levels of fibrinogen.[123, 125] This suggests that increased fibrinogen is a marker of IR, due to IL-6 activity. The odds of diabetes for our subjects in the highest quartile of fibrinogen decreased slightly, but remained significant after the adjustment of IR suggests fibrinogen can also be used as a marker for diabetes. It is widely understood that IR is necessary for type 2 diabetes, but not sufficient for diabetes.

C-reactive protein is the most studied inflammatory marker and is associated with diabetes.[30, 163] This association has also been shown in Japanese populations.[164] Unfortunately, our measurements of CRP were taken at exam 2, which occurred twenty years prior to Exam 4. Although CRP measurements taken 5 years apart are highly correlated, various diseases and metabolic changes occurring in that 20 year period would cause midlife CRP values to be highly inaccurate in predicting later-life inflammation.[165]

Studies have shown a modest correlation between CRP and fibrinogen in diabetic subjects.[166, 167] However, a subgroup analysis in our cohort showed a small, non-significant correlation between midlife CRP and fibrinogen ($r=0.06$, $P=0.08$) and the chi-square analysis showed a significant difference in the distribution of subjects by CRP and fibrinogen quartiles.

This study has some important strengths. To the best of our knowledge, this is the first study looking at the relationship between fibrinogen and diabetes in healthy Japanese elderly men. The utilization of the HAAS limits confounding by restricting subjects to Japanese men of the same age, ethnicity, sex-related factors and genetics. Including IR through the use of the McAuley index and HOMA index helps to elucidate the true relationship between fibrinogen and diabetes. The McAuley index is the only IR index utilizing triglyceride levels, and is best suited for epidemiological studies.[58]

However, we are aware of the limitations in this study. The restriction of the HAAS, while a strength, also limits the generalizability of our findings. A single measurement of fibrinogen prevented us from a longitudinal analysis of fibrinogen and diabetes. As a result, the cross-sectional nature of this study prevents us from drawing causal conclusions between fibrinogen and diabetes. It is unclear whether fibrinogen is a causal mechanism in the pathology of diabetes, or if fibrinogen is merely a marker of inflammation caused by diabetes. Also, due to recruitment and identification of participants using Census data and Selective Service registration records, selection bias is a possibility.

Our study shows an association between high fibrinogen levels and prevalent diabetes cases, after adjusting for major confounders. However, future studies using repeated measurements of fibrinogen and incident diabetes are needed to clarify the causal relationship between fibrinogen and diabetes.

Table 12. Selected Characteristics of Participants by Fibrinogen Quartile and Type 2 Diabetes Status: The Honolulu-Asia Aging Study

Variables	Fibrinogen Quartile 1 (≤264mg/L)	Fibrinogen Quartile 2 (265-296mg/L)	Fibrinogen Quartile 3 (297-338mg/L)	Fibrinogen Quartile 4 (≥339mg/L)	P	No Diabetes	Diabetes	P-Value
n=3,148	795	790	792	771		2246 (71.4%)	902 (28.6%)	
Age (years)	77.5 ± 4.2	78.0 ± 4.4	78.0 ± 4.4	78.0 ± 4.4	0.05	77.8 ± 4.4	77.9 ± 4.3	0.47
Waist Circumference (cm)	85.9 ± 8.7	85.6 ± 8.2	86.3 ± 8.9	86.4 ± 8.9	0.25	85.4 + 8.6	87.7 ± 8.6	<0.001
LDL Cholesterol (mg/dL)	102.4 ± 27.9	111.8 ± 30.0	115.4 ± 31.3	116.3 ± 31.7	<0.001	111.4± 30.6	110.8 ± 31.1	0.59
Prevalent CHD								
Yes	145 (4.60%)	136 (4.32%)	160 (5.08%)	186 (5.91%)	0.004	378 (12.00%)	249 (7.91%)	<0.0001
No	650 (20.64%)	654 (20.77%)	632 (20.07%)	586 (18.61%)		1,868 (59.32%)	654 (20.77%)	
Prevalent Hypertension (Y/N)								
Yes	575 (18.26%)	584 (18.55%)	593 (18.83%)	590 (18.74%)	0.28	1623 (51.54%)	719 (22.83%)	<0.001
No	220 (6.99%)	206 (6.54%)	199 (6.32%)	182 (5.78%)		623 (19.78%)	184 (5.84%)	
Smoking								
Never	308 (9.78%)	308 (9.78%)	316 (10.03%)	252 (8.03%)	0.002	834 (26.5%)	350 (11.1%)	0.06
Past	439 (13.94%)	438 (13.91%)	420 (13.34%)	442 (14.04%)		1236 (39.3%)	503 (16.0%)	
Current	48 (1.52%)	44 (1.40%)	56 (1.78%)	77 (2.45%)		176 (5.60%)	49 (1.60%)	
Alcohol Exam 2								
Non drinker	336 (10.67%)	338 (10.73%)	358 (11.37%)	31 (10.07%)	0.07	940 (29.9)	409 (13.0)	0.13
<1 a day	68 (2.16%)	61 (1.94%)	69 (2.19%)	63 (2.00%)		183 (5.8)	78 (2.5)	
1 to 2 a day	235 (7.46%)	265 (8.42%)	241 (7.65%)	225 (7.15%)		716 (22.7)	250 (8.0)	
3 or more	156 (4.95%)	126 (4.00%)	124 (3.94%)	166 (5.30%)		407 (12.9)	165 (5.2)	
HOMA								
Yes	502 (15.94%)	499 (15.85%)	542 (17.21%)	544 (17.28%)	0.002			
No	293 (9.30%)	291 (9.24%)	250 (7.94%)	228 (7.24%)				

Table 12. (Continued) Selected Characteristics of Participants by Fibrinogen Quartile and Type 2 Diabetes Status: The Honolulu-Asia Aging Study

Variables	Fibrinogen Quartile 1 (≤264mg/L)	Fibrinogen Quartile 2 (265-296mg/L)	Fibrinogen Quartile 3 (297-338mg/L)	Fibrinogen Quartile 4 (≥339mg/L)	P	No Diabetes	Diabetes	P-Value
McAuley								
Yes	290 (9.21%)	312 (9.91%)	335 (10.64%)	340 (10.80%)	0.01			
No	505 (16.04%)	478 (15.18%)	457 (14.51%)	432 (13.72%)				
Combined								
Yes	277 (8.80%)	305 (9.69%)	321 (10.19%)	333 (10.57%)	0.0072			
No	518 (16.45%)	485 (15.40%)	471 (14.96%)	439 (13.94%)				

Figure 10. Correlation between HOMA index and McAuley index

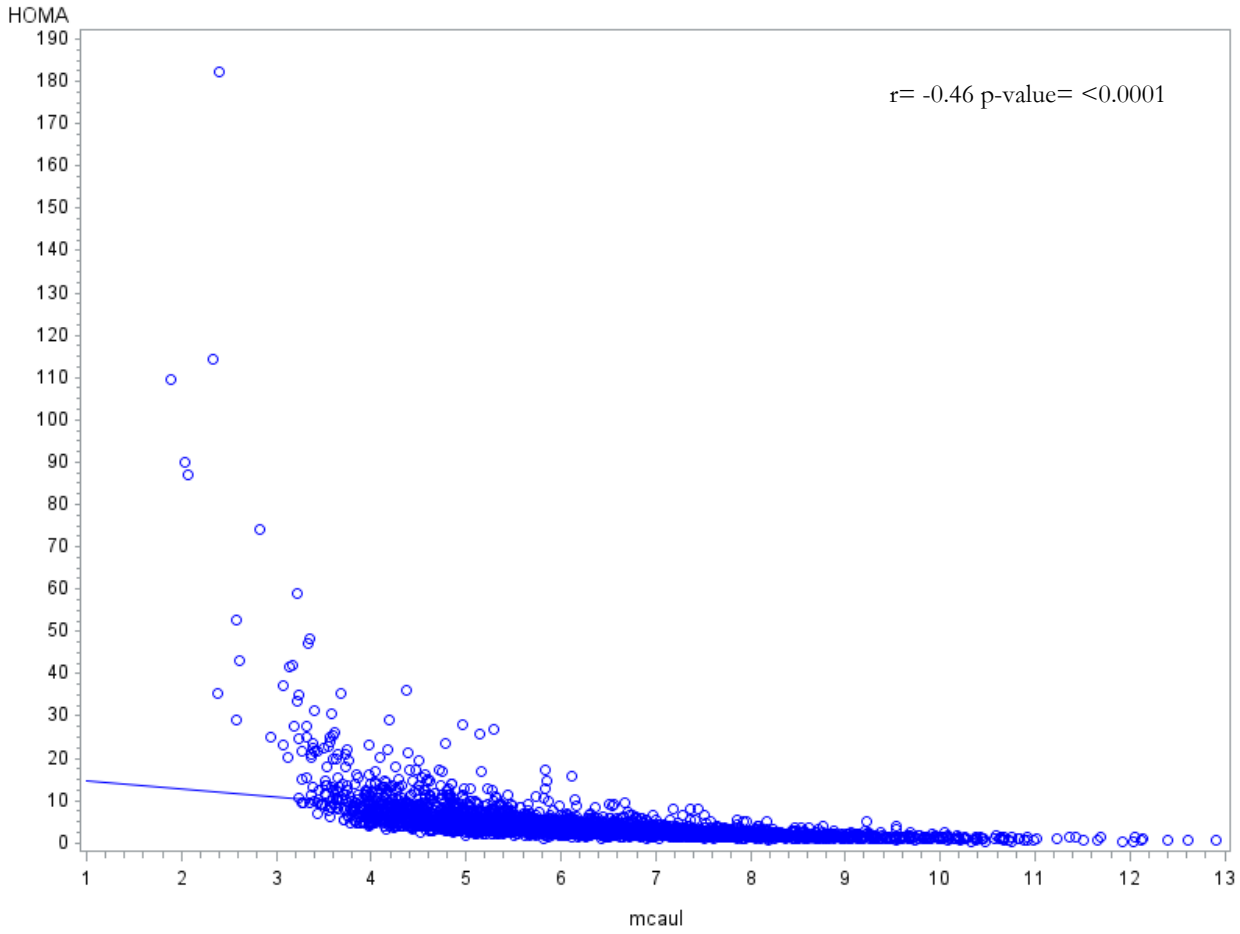


Figure 11. Correlation between midlife C-Reactive protein and later life fibrinogen

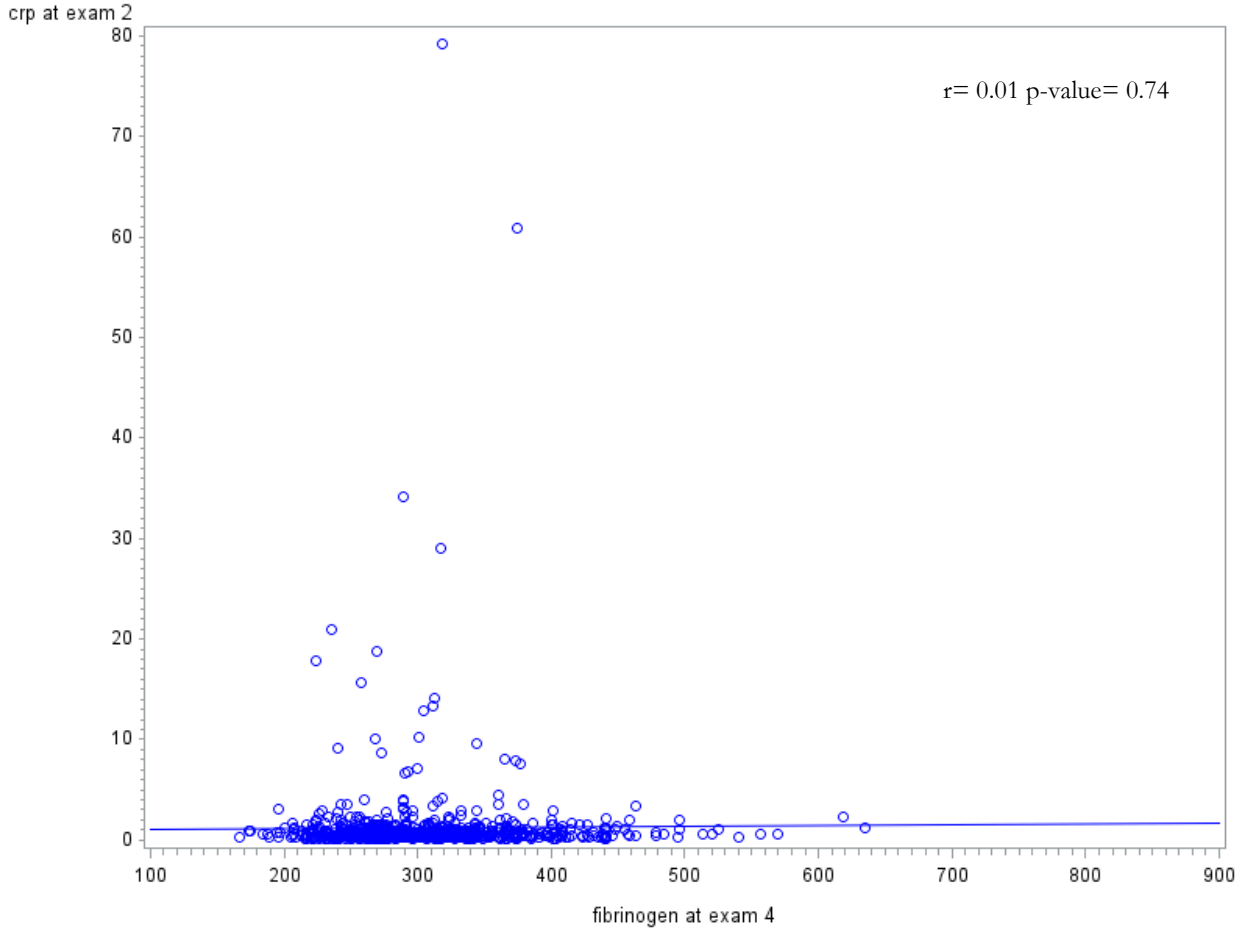


Figure 12. Correlation between later life fibrinogen and HOMA index

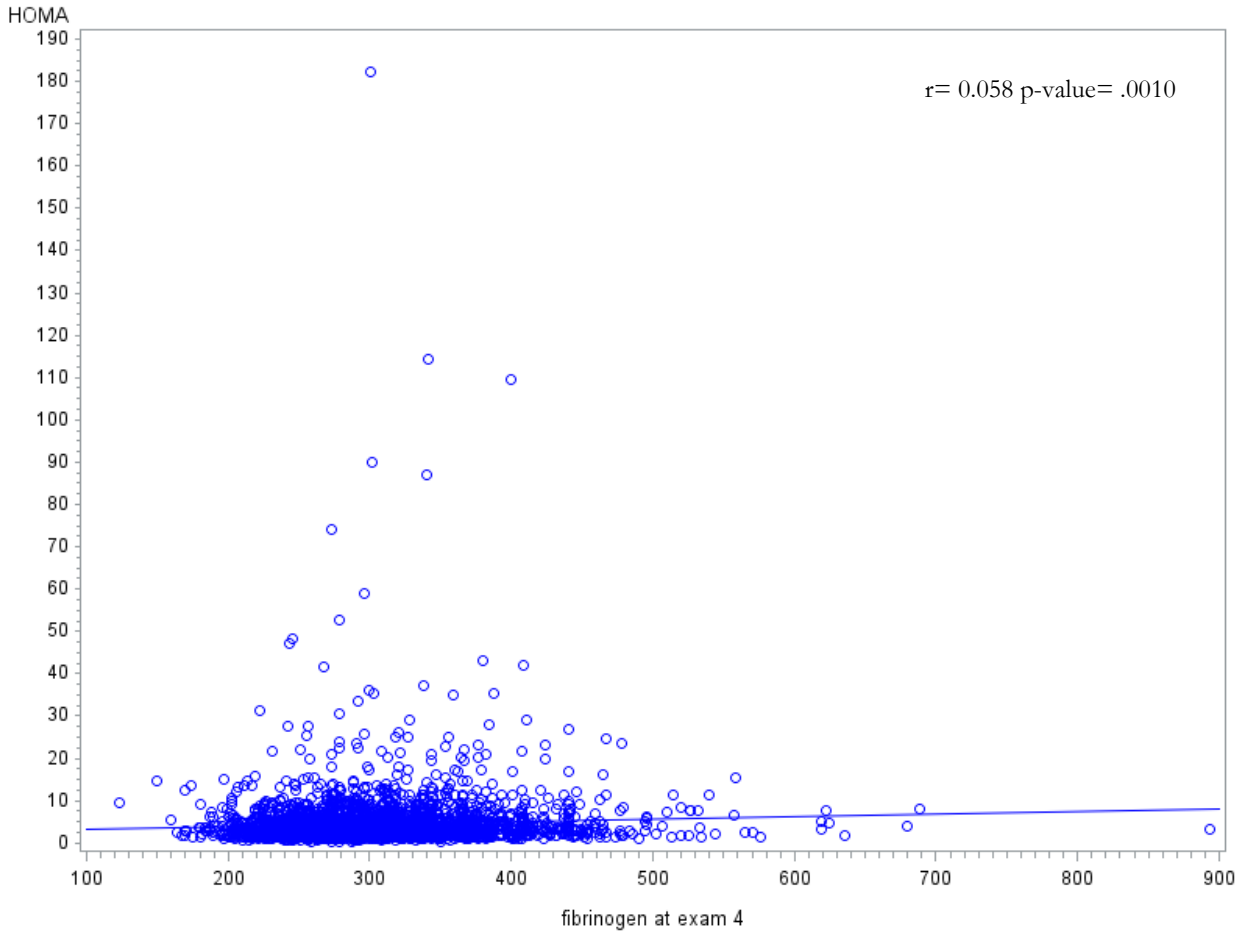


Figure 13. Correlation between later life fibrinogen and McAuley index

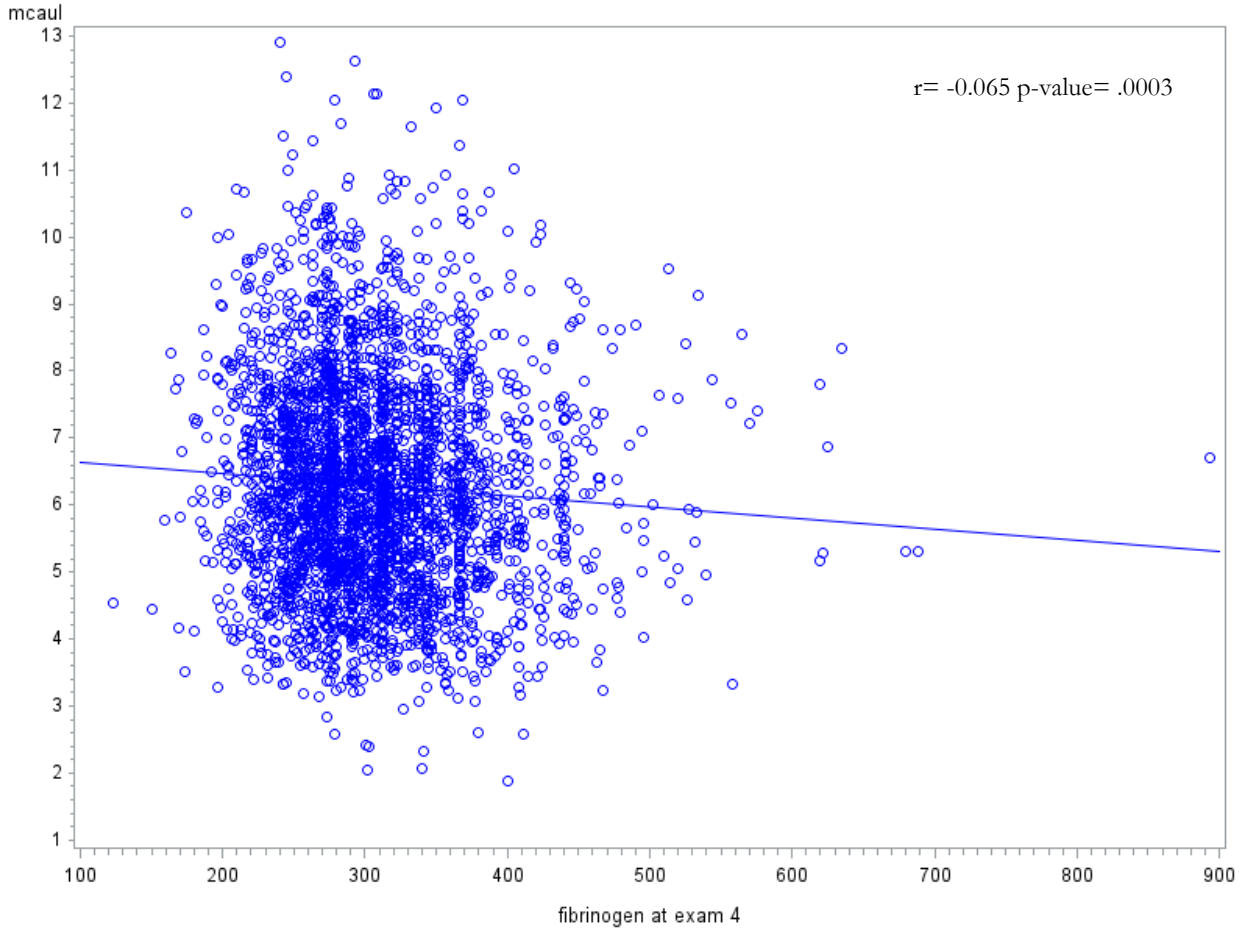


Table 13. Estimated adjusted associations (Odds Ratios [OR] and 95% confidence intervals [CI] of type 2 diabetes by fibrinogen quartiles using the HOMA/McAuley/Combined IR indices: results of multivariate logistic regression analysis

	Number of Cases of Diabetes	Unadjusted	Potential Confounders*	McAuley Index**	HOMA Index***	Combined Index****
Fibrinogen Quartile 1 (≤264) N=795	199	referent	referent	referent	referent	referent
Fibrinogen Quartile 2 (265 to 296) N=790	209	1.08 (0.86−1.4)	1.11 (0.88−1.40)	1.10 (0.86−1.36)	1.12 (0.88−1.40)	1.07 (0.85−1.35)
Fibrinogen Quartile 3 (297 to 338) N=792	239	1.30 (1.04−1.6)	1.31 (1.04−1.64)	1.25 (1.00−1.60)	1.27 (1.00−1.60)	1.25 (0.99−1.57)
Fibrinogen Quartile 4 (≥339) N=772	256	1.48 (1.2−1.8)	1.53 (1.22−1.91)	1.40 (1.11−1.76)	1.40 (1.11−1.77)	1.40 (1.11−1.76)
P for trend				p=0.0016	p=0.0016	p=0.0019

*Analyses were adjusted for age and waist circumference, LDL cholesterol, smoking, alcohol

**Analyses were adjusted for age, waist circumference, LDL cholesterol, prevalent hypertension, smoking, alcohol, coronary heart disease, and McAuley index

***Analyses were adjusted for age, waist circumference, LDL cholesterol, prevalent hypertension, smoking, alcohol, coronary heart disease, and HOMA Index

****Analyses were adjusted for age, waist circumference, LDL cholesterol, prevalent hypertension, smoking, alcohol, coronary heart disease, and Combined Index

Table 14. Estimated adjusted associations (Odds Ratios [OR] and 95% confidence intervals [CI] of type 2 diabetes by fibrinogen quartiles, stratified by three insulin resistance indices: results of logistic regression analysis

Stratification by Insulin Resistance*	Cases of Diabetes	Fibrinogen Quartile 1 (≤264)	Fibrinogen Quartile 2 (265 to 296)	Fibrinogen Quartile 3 (297 to 338)	Fibrinogen Quartile 4 (≥339)
McAuley Index					
Insulin Resistant (N=1,277)	498	referent	1.22 (0.87-1.72)	1.20 (0.86-1.68)	1.58 (1.14-2.20)
Not Insulin Resistant (N=1,872)	405	referent	0.94 (0.68-1.30)	1.30 (0.95-1.78)	1.23 (0.89-1.70)
HOMA Index					
Insulin Resistant (N=2,087)	764	referent	1.26 (0.96-1.64)	1.32 (1.02-1.72)	1.42 (1.09-1.84)
Not Insulin Resistant (N=1,062)	139	referent	0.72 (0.42-1.22)	1.058 (0.65-1.80)	1.48 (0.89-2.44)
Combined Index					
Insulin Resistant (N=1,236)	495	referent	1.20 (0.85-1.69)	1.20 (0.85-1.69)	1.54 (1.10-2.15)
Not Insulin Resistant (N=1,913)	408	referent	0.94 (0.68-1.30)	1.29 (0.94-1.76)	1.24 (0.90-1.71)

*Analyses were adjusted for age, waist circumference, smoking, alcohol, LDL cholesterol, prevalent hypertension and coronary heart disease

Table 15. Estimated adjusted associations (Odds Ratios [OR] and 95% confidence intervals [CI] of type 2 diabetes by fibrinogen quartiles using the HOMA/McAuley/Combined IR indices, stratified by hypertension: results of multivariate logistic regression analysis

Stratification by Hypertension *	Cases of Diabetes	Fibrinogen Quartile 1 (≤264)	Fibrinogen Quartile 2 (265 to 296)	Fibrinogen Quartile 3 (297 to 338)	Fibrinogen Quartile 4 (≥339)
McAuley Index					
Hypertensive (N=2,342)	719	referent	1.26 (0.96-1.64)	1.36 (1.05-1.78)	1.52 (1.16-1.98)
Not hypertensive (N=807)	184	referent	0.65 (0.40-1.08)	0.98 (0.61-1.58)	1.21 (0.75-1.94)
HOMA Index					
Hypertensive (N=2,342)	719	referent	1.29 (0.99-1.69)	1.36 (1.04-1.78)	1.52 (1.17-1.78)
Not hypertensive (N=807)	184	referent	0.68 (0.41-1.13)	1.03 (0.64-1.68)	1.17 (0.72-1.91)
Combined Index					
Hypertensive (N=2,342)	719	referent	1.25 (0.95-1.63)	1.37 (1.05-1.78)	1.51 (1.16-1.96)
Not hypertensive (N=807)	184	referent	0.65 (0.39-1.07)	0.97 (0.60-1.56)	1.19 (0.74-1.93)

*Analyses were adjusted for age, waist circumference, smoking, alcohol, LDL cholesterol, Insulin resistance, and coronary heart disease

Table 16. Estimated adjusted associations (Odds Ratios [OR] and 95% confidence intervals [CI] of type 2 diabetes by fibrinogen quartiles using the HOMA/McAuley/Combined IR indices, stratified by coronary heart disease: results of multivariate logistic regression analysis

Stratification by CHD *	Cases of Diabetes	Fibrinogen Quartile 1 (≤264)	Fibrinogen Quartile 2 (265 to 296)	Fibrinogen Quartile 3 (297 to 338)	Fibrinogen Quartile 4 (≥339)
McAuley Index					
CHD (N=627)	249	referent	1.00 (0.61-1.65)	1.31 (0.81-2.11)	1.32 (0.83-2.01)
No CHD (N=2,522)	654	referent	1.09 (0.83-1.42)	1.20 (0.92-1.57)	1.42 (1.09-1.86)
HOMA Index					
CHD (N=627)	249	referent	1.02 (0.61-1.71)	1.44 (0.88-2.35)	1.32 (0.82-2.12)
No CHD (N=2,522)	654	referent	1.13 (0.87-1.48)	1.19 (0.91-1.56)	1.44 (1.10-1.88)
Combined Index					
CHD (N=627)	249	referent	0.99 (0.60-1.64)	1.30 (0.80-2.09)	1.31 (0.82-2.08)
No CHD (N=2,522)	654	referent	1.08 (0.83-1.40)	1.21 (0.92-1.57)	1.41 (1.08-1.84)

*Analyses were adjusted for age, waist circumference, smoking, alcohol, LDL cholesterol, and prevalent hypertension

Chapter 5

Discussions and Conclusions

To better aide the reader, the final chapter of this dissertation will briefly summarize the objectives of each research question and findings. However, the majority of this chapter will be spent discussing implication of the results and possible next steps.

Summary of the Research Questions

With the continued advancement of medicine and technology, the prevalence and incidence of diseases associated with increased life expectancy will increase. As a result, two of the most common types of chronic diseases, neurodegenerative diseases and metabolic diseases, will continue to add to the global health burden.

Recent research has identified insulin signaling as an integral part of AD/dementia disease pathology. Epidemiologic research has mainly focused on associations between type 2 diabetes and AD/dementia. Few studies, however, have examined whether IR is associated with these two cognitive diseases. The first study of this dissertation focuses on whether late-life IR in Japanese American men is associated with AD/dementia. The second and third studies focus on the relationship between fibrinogen, IR and type 2 diabetes.

Insulin Resistance, AD/dementia Study

Original hypothesis: Insulin resistance is positively associated with increased risk of AD/dementia.

Conclusions: Insulin resistance may be inversely associated with AD/dementia risk.

Conclusions

Based on previous literature which demonstrated a positive association between diabetes and neurological decline, I hypothesized that I would find a positive association between subjects with IR and odds of AD/dementia. However, results obtained in the current study were the opposite of what was predicted. Subjects who were the identified as IR according to the HOMA model, the highest quartile, had lower odds of AD/dementia, compared to the referent quartile. This protective effect of IR was consistent

when using the McAuley index of IR. Subjects who were in the bottom two quartiles based on the McAuley index had lower OR for AD/dementia.

In addition to our main findings, our studies confirmed that carriers of the APOE ε4 allele had a 50% increased odds of dementia and 59% increased odds of AD. These findings did not change when using the HOMA index or McAuley index. While the results after stratifying by APOE ε4 allele status were not statistically significant, the difference in the OR between carriers of that allele and non-carriers suggest further research to clarify whether APOE ε4 allele modifies the effect of IR on AD/dementia.

Discussion, Implications, and Next Steps

My main findings are quite exciting because few studies have looked at the relationship between later-life IR and AD/dementia, and none have investigated that in Japanese men using the HOMA-IR index or the McAuley index. While studies have found that midlife IR is associated with an increased risk of incident AD, the inverse associations discovered in this study may shed some light into how aging may affect chronic disease relationships.[46, 47]

Typically, IR is assumed to be a precursor to type 2 diabetes and contributors to the metabolic syndrome, both widely accepted chronic diseases. However, in older adults, states of hyperinsulinemia may not be harmful, and in some cases, beneficial. Hyperinsulinemia stimulates skeletal muscle protein anabolism in the elderly and patients in an induced hyperinsulinemic state see an improvement in memory tests.[88, 92, 168] Results from these studies support our findings that IR may be protective against dementia and AD in Japanese men.

Further research is needed to examine the relationship between later-life IR and cognitive diseases in both men and women. Due to lack of data at some exams, the conclusions drawn from this study are limited. It would be beneficial to collect IR data from multiple time points, starting from midlife and ending at later life, to fully examine the longitudinal relationship between IR and AD/dementia.

Fibrinogen and Insulin Resistance Study

Original hypothesis: Increased fibrinogen levels are associated with increased odds of insulin resistance.

Conclusions: Findings support original hypothesis.

Conclusions

The findings from this study add to the small, but growing literature associating IR with fibrinogen. Results from Japanese only studies provide mixed results. In this cohort of Japanese men, subjects who were in the third quartile of fibrinogen saw significantly increased odds of IR. However, subjects in the highest quartile also saw a 23% increase in odds, and the CI very narrowly missed statistical significance. This indicates a strong possibility that subjects who fall in both the third and fourth quartiles would be at increased odds of IR. Additionally in this study, I observed increased alcohol consumption led to a decreased odds of IR. Also, subjects who had increased waist circumference or who were hypertensive were at increased odds of IR.

Discussion, Implications, and Next Steps

Insulin resistance is an important risk factor for type 2 diabetes. As such, finding an effective method to screen for IR can have positive public health utility. Ideally, identifying earlier biomarkers to predict increased risk of IR would have a greater impact on developing strategies to decrease incidence of IR. Due to the limitations of this study, it was not possible to investigate midlife fibrinogen values and incident IR. As medicine and medical technology continue to advance, life expectancy will also increase. Consequently, people who develop IR in later life will benefit from research that studies IR as a late life biomarker, versus its implications at midlife. Future studies would benefit from investigating the relationship of fibrinogen with IR, and whether changes in fibrinogen concentrations alters disease progression as a person ages into their 70's and 80's.

Fibrinogen and Type 2 Diabetes Study

Original hypothesis: Increased fibrinogen levels are associated with increased odds of type 2 diabetes.

Conclusions: Findings support original hypothesis.

Conclusions

Increased fibrinogen concentrations were significantly associated with increased odds of diabetes. Subjects who were in the highest quartile of fibrinogen had a 40% increased odds of diabetes, even after the adjustment of IR. Subjects in the third quartile also had an increased odds of at least 25%, but the CI included 1 (95% CI 1.0, 1.6). But is highly likely that subjects in the third quartile are still at increased odds of diabetes. A significant p for trend was found in the final multi-variate model, regardless of which IR index was used. Subjects who were hypertensive or who had CHD had an increased odds of around 30% and around 70% of diabetes respectively.

Discussion, Implications, and Next Steps

Fibrinogen's association with type 2 diabetes is not well studied as the majority of research has focused on CRP and diabetes. Prior studies found that fibrinogen's association with diabetes disappeared after adjusting for either IR or body fat. However, this study shows that even after adjusting for IR, and also CHD, hypertension and waist circumference, fibrinogen is still associated with an increased odds of diabetes. Fibrinogen and CRP have been shown in previous studies($r=0.44$, p-value=<0.05) to have a modest correlation.[169] Future studies should first confirm the relationship between fibrinogen and CRP levels as a population ages. Secondly, it would also add to the literature to study whether fibrinogen and CRP have similar associations with diabetes. Due to limitations of the data set, these comparisons could not be made in the current study as CRP was measured at midlife in the HAAS and fibrinogen was only measured at later life.

Tying it all together

The purpose of the first study may seem disconnected from the other two studies, however, from a public health standpoint, connections can be drawn to relate the three studies. One of the goals of public health is to prevent the onset of diseases. Many times, public health practices turn to screening and education to achieve that goal. While fibrinogen's mechanistic role in IR and diabetes is still unclear, its use as an inflammatory biomarker can aid a physician when diagnosing a patient.

Fibrinogen has been found to be associated with increased odds for IR and diabetes in older Japanese Americans. In these same subjects, those that were IR had a decreased odds of dementia and AD. While future studies are needed, it is possible that fibrinogen levels can be used as a marker of AD/dementia. Previous studies have found higher levels of fibrinogen in older adults result in increased risk of dementia.[170, 171] But, these studies only adjusted for diabetes, and not for IR.

The use of the HOMA index and McAuley index as proxies of IR revealed subtle differences in the magnitude of the odd ratio estimates as well as the width of the confidence intervals. Studies have mixed results regarding the validity of HOMA versus McAuley as indicators of IR compared to the gold standard of the hyperinsulinemic euglycemic clamp technique.[57, 172] A recent study published in 2015 by Gutch et al. supports the use of the McAuley index as a better index for IR in an epidemiologic study in populations that are normal glycemic, which is true for my study populations where I examined fibrinogen.[58] All three of my studies utilized a novel method of assessing IR by combining results from both the HOMA index and McAuley index to increase the sensitivity of IR status. It would be beneficial for future research to continue to validate the use of the McAuley index, HOMA index, and the Combined index in Japanese populations, compared to the gold standard.

The study of chronic diseases in older adults is often times less straight forward than in adults in midlife. This is evident by my findings that IR may be protective for AD/dementia. Also, it is well known how both infectious and chronic diseases affect the body's inflammatory response, and therefore cause changes in the concentrations of inflammatory markers. The human body's response to IR, diabetes, AD, or dementia at midlife may differ from responses in later life. Additionally, researchers must also consider how competing diseases would affect the associations drawn from the three studies. While the current studies attempted to account for competing risks by CHD, hypertension, diabetes, and the APOE ε4 allele, each of those factors may play different mechanistic roles in IR, diabetes, dementia and AD. Also, it is unclear whether the observed inflammation is strictly a result of the studied markers, or just an effect of aging. Therein lies the danger of using specific cutoff values determined from one study and applying it to studies of differing populations. It is tempting as an epidemiologist, to want to identify the magic cutoff value, but for

the reasons listed above researchers should be deterred from making such claims. However, this does not take away from the value of the findings from these three studies.

In conclusion, the results from these three studies should spur future studies to confirm whether IR is inversely associated with AD/dementia and whether fibrinogen is associated with both IR and diabetes. Public Health prevention efforts directed towards diabetes, IR, dementia and AD should include confirming whether inflammatory markers are risk factors for incident cases. However, it is equally as important to understand how such inflammatory markers are associated with those diseases at later life.

Office of Research Compliance
Human Studies Program

UNIVERSITY of HAWAI'I MĀNOA

March 29, 2016

TO: Thomas Lee
Andrew Grandinetti, Ph.D.
Principal Investigators
Public Health

FROM: Denise A. Lin-DeShetler, MPH, MA
Director

SUBJECT: CHS #23928 - "Alzheimer's and Dementia in the Honolulu Asia Aging Study: The Role of C-reactive Protein and Insulin Resistance"

This letter is your record of the Human Studies Program approval of this study as exempt.

On March 29, 2016, the University of Hawai'i (UH) Human Studies Program approved this study as exempt from federal regulations pertaining to the protection of human research participants. The authority for the exemption applicable to your study is documented in the Code of Federal Regulations at 45 CFR 46.101(b) (Category 4).

Exempt studies are subject to the ethical principles articulated in The Belmont Report, found at http://www.hawaii.edu/irb/html/manual/appendices/A/belmont.html

Exempt studies do not require regular continuing review by the Human Studies Program. However, if you propose to modify your study, you must receive approval from the Human Studies Program prior to implementing any changes. You can submit your proposed changes via email at uhirb@hawaii.edu. (The subject line should read: Exempt Study Modification.) The Human Studies Program may review the exempt status at that time and request an application for approval as non-exempt research.

In order to protect the confidentiality of research participants, we encourage you to destroy private information which can be linked to the identities of individuals as soon as it is reasonable to do so. Signed consent forms, as applicable to your study, should be maintained for at least the duration of your project.

This approval does not expire. However, please notify the Human Studies Program when your study is complete. Upon notification, we will close our files pertaining to your study.

If you have any questions relating to the protection of human research participants, please contact the Human Studies Program at 956-5007 or uhirb@hawaii.edu. We wish you success in carrying out your research project.

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