Obesity and dietary habits across the lifespan and risk of multiple myeloma and its precursor

Maríanna Þórðardóttir

Thesis for the degree of Philosophiae Doctor

Supervisor: Sigurður Yngvi Kristinsson

Doctoral committee: Sigurður Yngvi Kristinsson, Sigrún Helga Lund, Laufey Steingrímsdóttir, Vilmundur Guðnason og Ola Landgren

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Holdafar og mataræði á mismunandi æviskeiðum og tengsl við mergæxli og forstig þess

Maríanna Þórðardóttir

Ritgerð til doktorsgráðu

Leiðbeinandi: Sigurður Yngvi Kristinsson

Doktorsnefnd: Sigurður Yngvi Kristinsson, Sigrún Helga Lund, Laufey Steingrímsdóttir, Vilmundur Guðnason og Ola Landgren

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Ágrip

Inngangur: Mergæxli er ólæknandi krabbamein í beinmerg sem upprunnið er í mótefnamyndandi plasmafrumum. Forstig mergæxlis kallast góðkynja einstofna mótefnahækkun (e. monoclonal gammopathy of undetermined significance, MGUS) og einkennist af hækkun á einstofna mótefni í blóði án þess að skilmerkjum um mergæxli eða aðra tengda sjúkdóma sé að ræða. Orsök MGUS og mergæxlis er að miklu leyti óþekkt en þekktir áhættuþættir eru aldur, kyn, kynþáttur og fjölskyldusaga. Mergæxli bættist nýlega í hóp þeirra krabbameina sem talið er að offita hafi áhrif á en rannsóknir á áhrifum annarra lífsstílstengdra þátta á mergæxli eru fáar og eru niðurstöður þeirra ekki afgerandi. Meginmarkmið þessarar ritgerðar var að kanna áhrif offitu og mataræðis á MGUS og framþróun yfir í mergæxli og tengda sjúkdóma.

Efniviður og aðferðir: Þrjár vísindagreinar liggja að baki þessari ritgerð og notast var við gögn úr Öldrunarrannsókn Hjartaverndar (N=5.764). Öldrunarrannsóknin, sem hófst árið 2002 og lauk í byrjun árs 2006, er framhaldsrannsókn af hinni lýðgrunduðu Reykjavíkurrannsókn sem hófst árið 1967. Í Öldrunarrannsókninni var miklum upplýsingum um þátttakendur safnað. Meðal annars voru framkvæmdar ýmsar mælingar á holdafari, upplýsingum safnað um mataræði á þremur mismunandi æviskeiðum og tekin blóðsýni. Árið 2013 skimuðum við fyrir MGUS og MGUS af völdum léttra keðja (LC-MGUS) og notuðum til þess fyrrnefnd blóðsýni. Þátttakendum var fylgt eftir til ársins 2014 og upplýsingum um mergæxli og tengda sjúkdóma var safnað úr Krabbameinsskrá. Í **rannsókn I** skoðuðum við samband 11 holdafarsmælinga og MGUS og LC-MGUS. Til viðbótar þá skoðuðum við hvort niðurstöður þessara mælinga tengdust aukinni áhættu á framþróun úr MGUS/LC-MGUS yfir í mergæxli og tengda sjúkdóma. Í **rannsókn II** skoðuðum við mataræði á þremur mismunandi æviskeiðum; á unglingsárum (14-19 ára), á miðjum aldri (40-50 ára) og á eldri árum (við upphaf Öldrunarrannsóknarinnar), og samband þess við MGUS og LC-MGUS (unglingsár og miður aldur) og framþróun yfir í mergæxli og tengda sjúkdóma (eldri ár). Í **rannsókn III** var notast við meginþáttagreiningu (e. principal component analysis) til að bera kennsl á fæðumynstur á unglingsárunum. Við skoðuðum samband þessara fæðumynstra við MGUS og LC-MGUS. Við notuðum lógístíska aðhvarfsgreiningu þegar samband útsetninga við MGUS og LC-MGUS var kannað en Cox-líkan til að skoða áhrif útsetninga á framþróun úr MGUS/LC-MGUS yfir í mergæxli og tengda sjúkdóma.

Niðurstöður: Meðalaldur þátttakenda var 77 ár (spönn 66-98). Alls greindust 300 (5.2%) einstaklingar með MGUS og 275 (4.8%) með LC-MGUS, þar af

greindust 18 með mergæxli og 11 til viðbótar með aðra tengda sjúkdóma á eftirfylgdartímanum, sem var að meðaltali var 8 ár. Í **rannsókn I** fannst ekkert samband á milli holdafarsmælinganna og MGUS eða LC-MGUS en við fundum að þeir sem voru með háan líkamsþyngdarstuðul (≥ 25 kg/m^2) á miðjum aldri voru í aukinni áhættu á framþróun úr MGUS/LC-MGUS yfir í mergæxli og tengda sjúkdóma (hazard ratio [HR] = 2.66, 95% confidence interval [CI] 1.17-6.05). Í **rannsókn II** fundust neikvæð tengsl á milli MGUS og ávaxtaneyslu á unglingsárum (odds ratio [OR] = 0.62, 95% CI 0.41-0.95) og neyslu á heilhveitibrauði á miðjum aldri (OR = 0.75, 95% CI 0.57-0.99). Til viðbótar þá fundum við að aukin ávaxtaneysla á eldri árum minnkaði áhættuna á framþróun úr MGUS/LC-MGUS yfir í mergæxli og tengda sjúkdóma (HR = 0.45, 95% CI 0.21-0.96). Tvö fæðumynstur mynduðust í meginhlutagreiningunni í **rannsókn III**, annað einkenndist af mikilli inntöku af söltuðu og reyktu kjöti og fiski, innmat, rúgbrauði, mjólk, hafragraut og kartöflum (gamla íslenska mataræðið) en hitt einkenndist af mikilli inntöku af ávöxtum, grænmeti og fiski sem álegg eða í salat. Niðurstöðurnar sýndu að þeir sem skoruðu hátt á fyrra fæðumynstrinu voru ólíklegri til að greinast með MGUS (OR = 0.68, 95% CI 0.50-0.93) og LC-MGUS (OR = 0.67, 95% CI 0.49-0.92) síðar á ævinni.

Ályktun: Niðurstöður þessara lýðgrunduðu rannsókna benda til þess mataræði spili hlutverk bæði í þróun MGUS og framþróun yfir í mergæxli og tengda sjúkdóma. Einnig benda þær til þess að líkamsþyngdarstuðull á miðjum aldri sé áhættuþáttur fyrir framþróun úr MGUS/LC-MGUS yfir í mergæxli og tengda sjúkdóma. Þessar niðurstöður gefa til kynna að hegðunar- og umhverfisþættir eigi þátt í þróun mergæxlis og undirstrika mikilvægi þess að taka heilsusamlegar ákvarðanir er varða lífsstíl.

Lykilorð:
Offita, mataræði, forstig mergæxlis, mergæxli, lýðgrunduð rannsókn.

Abstract

Background and aim: Multiple myeloma is a malignant chronic B-cell disorder characterized by a monoclonal proliferation of plasma cells in the bone marrow. Almost all multiple myeloma cases are preceded by the premalignant state, monoclonal gammopathy of undetermined significance (MGUS). The etiology of multiple myeloma and MGUS is to a large extent unknown, with age, sex, race, and family history as the most established risk factors. Obesity was recently acknowledged as the first identified modifiable risk factor for multiple myeloma, but results on other lifestyle-related factors, such as diet, are scarce and inconclusive. The overall aim of this thesis was to examine the impact of obesity and diet on MGUS as well as its effect on progression from MGUS to multiple myeloma and other lymphoproliferative diseases.

Materials and methods: Three papers form the foundation for this thesis, using data from the population-based AGES-Reykjavik Study (N=5,764). The AGES-Reykjavik Study is a continuation of the population-based Reykjavik Study (1967-1996). It was initiated in 2002 and recruitment ended in 2006. Extensive data on numerous health related factors were collected, including various obesity assessments, diet history throughout the lifespan, and blood samples. In 2013 we screened all participants for MGUS and light-chain MGUS (LC-MGUS) in serum, using samples collected at study entry (2002-2006). The participants were followed prospectively from AGES-Reykjavik Study entry to March 2014 and information on multiple myeloma and other lymphoproliferative diseases was collected from the Icelandic Cancer Registry. In **study I** we analyzed the association between 11 obesity measures and MGUS and LC-MGUS. We additionally analyzed its association with progression from MGUS/LC-MGUS to multiple myeloma and other lymphoproliferative diseases. In **study II** we analyzed dietary intake from three different periods in life; adolescence (14-19 years), midlife (40-50 years), and late life intake at study entry, and its association with MGUS and LC-MGUS (adolescence and midlife) and progression from MGUS/LC-MGUS to multiple myeloma and other lymphoproliferative diseases (late life). In **study III** we performed principal component analyzes to identify dietary patterns in adolescence and tested its association with MGUS and LC-MGUS. We used logistic regression when analyzing the associations between the exposures and MGUS/LC-MGUS and Cox proportional-hazard regression to test the effect of the exposures and risk of progression from MGUS/LC-MGUS and multiple myeloma and other lymphoproliferative diseases.

Results: Mean age of participants was 77 years (range 66-98). We identified 300 (5.2%) individuals with MGUS and 275 (4.8%) with LC-MGUS and during a medium of 8 years of follow-up, 18 of them progressed to multiple myeloma and 11 to other lymphoproliferative diseases. In **study I** we found none of our obesity exposures to be associated with MGUS or LC-MGUS, however we found that high midlife BMI (≥ 25 kg/m^2), derived from the Reykjavik Study data, increased risk of progressing from MGUS/LC-MGUS to multiple myeloma and other lymphoproliferative diseases (hazard ratio [HR] = 2.66, 95% confidence interval [CI] 1.17-6.05). In **study II** we found that adolescent fruit intake and midlife whole wheat bread intake was inversely associated with MGUS (odds ratio [OR] = 0.62, 95% CI 0.41-0.95 and OR = 0.75, 95% CI 0.57-0.99, respectively) and that late life fruit intake reduced risk of progressing from MGUS/LC-MGUS to multiple myeloma and other lymphoproliferative diseases (HR = 0.45, 95% CI 0.21-0.96). In **study III** we identified two dietary patterns and labelled them, based on their intake characteristics, as Traditional early 20th century diet (salted or smoked meat and fish, blood and liver sausage, rye bread, milk and milk products, oatmeal, potatoes) and Healthy diet (fruit, vegetables, fish on bread or in salad). We found that the Traditional early 20th century diet was inversely associated with both MGUS and LC-MGUS (OR = 0.68 95% CI 0.50-0.93 and OR = 0.67, 95% CI 0.49-0.92, respectively).

Conclusion: The results of our studies suggest that diet plays a role in the etiology of MGUS and multiple myeloma. They additionally suggest that BMI is a risk factor for progression from MGUS/LC-MGUS to multiple myeloma and related diseases. These findings indicate a potential role of behavioral and environmental factors in myelomagenesis and that the risk of multiple myeloma can be modified by taking favorable lifestyle related decisions.

Keywords:
Obesity, diet, monoclonal gammopathy of undetermined significance, multiple myeloma, population-based data.

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Contents

List of abbreviations

AGES	Age, Gene/Environment Susceptibility Study
BMI	Body mass index
CI	Confidence interval
CT	Computed tomography
FFQ	Food frequency questionnaire
FLC	Free light chain
HR	Hazard ratio
Ig	Immunoglobulin
IHA	Icelandic Heart Association
LC-MGUS	Light-chain monoclonal gammopathy of undetermined significance
MGUS	Monoclonal gammopathy of undetermined significance
OR	Odds ratio
PCA	Principal component analysis
SEER	Surveillance, Epidemiology, and End Results
SPEP	Serum protein electrophoresis
WHO	World Health Organization

List of figures

List of tables

List of original papers

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals (I-III):

I. **Obesity and risk of monoclonal gammopathy of undetermined significance and progression to multiple myeloma: a population-based study.** Thordardottir M, Lindqvist EK, Lund SH, Costello R, Burton D, Korde N, Mailankody S, Eiriksdottir G, Launer LJ, Gudnason V, Harris TB, Landgren O, and Kristinsson SY. *Blood Advances*. 2017;1(24):2186-92.

II. **Dietary intake is associated with risk of multiple myeloma and its precursor disease**. Thordardottir M, Lindqvist EK, Lund SH, Costello R, Burton D, Steingrimsdottir L, Korde N, Mailankody S, Eiriksdottir G, Launer LJ, Gudnason V, Harris TB, Landgren O, Torfadottir JE, Kristinsson SY*. PloS one*. 2018;13(11)

III. **Adolescent diet and risk of monoclonal gammopathy of undetermined signficance**. Thordardottir M, Birgisdottir BE, Steingrimsdottir L, Lindqvist EK, Aspelund T, Costello R, Burton D, Korde N, Mailankody S, Eiriksdottir G, Launer LJ, Harris TB, Landgren O, Gudnason V, Kristinsson SY, and Torfadottir JE*. Submitted.*

In addition, some unpublished data may be presented:
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Declaration of contribution

I, Maríanna Þórðardóttir, planned the research work for the papers that form the foundation for this thesis in close collaboration with my supervisor. I conducted all statistical analysis for papers I-III in collaboration with my supervisor and a statistician. I also drafted all manuscripts and revised in close collaboration with my co-authors. I wrote this thesis with the solid guidance of my supervisor and doctoral committee.

1 Introduction

According to the World Health Organization (WHO) cancer is globally the second leading cause of death, and is estimated to be responsible for approximately 9.6 million deaths worldwide in 2018 [1]. The most frequent cancer types differ between the sexes, but overall lung, breast, and colorectal are the most common cancers whereas lung, colorectal, and stomach cause the most cancer deaths worldwide [1]. Multiple myeloma is the second most common blood cancer in the world [2], after lymphoma, and accounts for 10% of all hematologic malignancies [3]. Compared to other types of cancer multiple myeloma is relatively rare and accounts for 1.8% and 1.2% of all new cancer cases in the United States and the Nordic countries, respectively [4, 5]. Although it is rare, it is more common among men than women and among individuals of African descent [5, 6]. Almost all cases of multiple myeloma are preceded by the premalignant asymptomatic condition monoclonal gammopathy of undetermined significance (MGUS) [7, 8].

Adoption of behaviors and lifestyle factors known to cause cancer has increased the burden of cancer worldwide, and is expected to further increase it [9]. WHO estimated that 30-50% of cancer deaths are due to behavioral and lifestyle factors; high body mass index (BMI), physical inactivity, use of tobacco and alcohol, and poor diet, and can therefore be preventable [1]. Obesity was recently acknowledged as the first modifiable risk factor for multiple myeloma [10], but few established non-modifiable risk factors have been previously identified [6, 11-14]. Poor diet has been found to be associated with many different types of cancers, inverse associations with intake of fruit and vegetables, fish, and whole grains have been found, while red and processes meat is believed to increase the risk [15, 16]. However, the literature regarding the association between diet and multiple myeloma is scarce and inconclusive [17-23].

There is a gap in the literature with regards to preventable risk factors for multiple myeloma and its precursor condition, MGUS. This thesis focuses on lifestyle related factors, with emphasis on obesity and diet, and risk of MGUS and progression to multiple myeloma and other related diseases. Understanding the etiology of these diseases, especially identifying possible modifiable risk factors, could have a major public health impact.

1.1 Multiple myeloma

Multiple myeloma is a chronic B-cell disorder characterized by a monoclonal proliferation of plasma cells (white blood cells) in the bone marrow, which often results in low blood counts, renal failure, and bone loss [24]. The disease affects most commonly locations of active bone marrow in adults such as the bones of the spine, pelvis, rib cage, skull, shoulders, and hips [2].

Each type of plasma cell makes a different type of antibody that are a part of the immune system and play a role in fighting infections. In multiple myeloma a plasma cell becomes abnormal and starts to make a copy of itself. The proliferation of the plasma cell results in overproduction of monoclonal immunoglobulins (Ig), known as M-proteins, that can be detected on serum protein electrophoresis (SPEP). Each type of M-protein consists of pairs of heavy chain (IgG, IgA, IgM, IgE, or IgD) and light chain (kappa or lambda) (Figure 1) [24, 25].

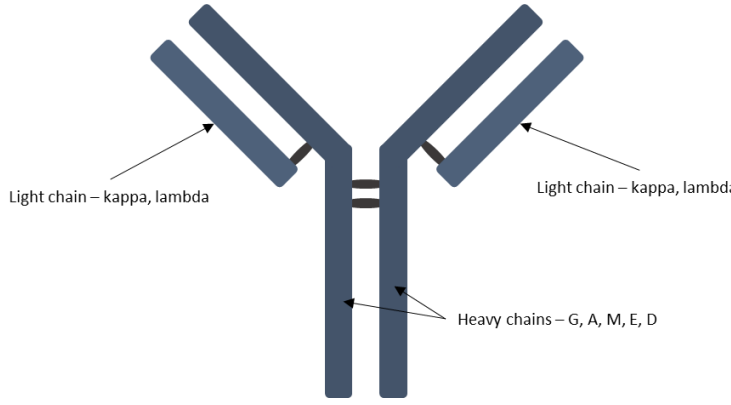

Figure 1 Immunoglobulin

The clinical signs and symptoms of multiple myeloma patients are usually caused by the monoclonal products or by direct tumor growth, but approximately 14% of all multiple myeloma patients are asymptomatic at diagnosis [26] . Bone pain is the most common symptom in patients, anemia is present in about 70-80% of patients, renal failure in about 50%, and hypercalcemia occurs in about one fourth of patients [3, 27].

The diagnostic criteria for multiple myeloma are:

≥10% clonal plasma cells in the bone marrow or biopsy-proven plasmacytoma.
Evidence of end organ damage, specifically:

- Hypercalcemia
- Renal insufficiency
- Anemia
- Bone lesions

Any one or more of the following biomarkers of malignancy:

- Clonal bone marrow plasma cell percentage ≥60%
- Serum free light chain (FLC) ratio ≥100
- >1 focal lesions on MRI [24].

Smoldering multiple myeloma (asymptomatic multiple myeloma) is an intermediate stage between MGUS and active multiple myeloma and the diagnostic criteria are (both criteria must be met):

Serum monoclonal protein ≥ 30 g/L and/or clonal plasma cells 10-60%.
Absence of myeloma defining events (end organ damage and biomarkers of malignancy as described above) or amyloidosis [24].

1.1.1 Incidence

Multiple myeloma is a disease of the elderly, with a median age at diagnosis at 70-71 years, and is only occasionally diagnosed in patients below 40 years of age [5, 28]. The incidence of multiple myeloma is higher in men than in women [5, 28], and it is more common in individuals of African descent compared to other races [5, 6]. According to Global Cancer Statistics 2018 the worldwide age-standardized incidence of multiple myeloma was 2.1 per 100.000 and 1.4 per 100.000 for men and women, respectively [29]. In 2016, the age-standardized incidence of multiple myeloma in Iceland was 4.3 per 100,000 for men and 3.6 per 100,000 for women (Figure 2), similar to that of other Nordic countries [4, 30]. The reason for the low worldwide incidence when compared to the Nordic countries is because there is a great geographical difference with regards to cancer incidence. The worldwide incidence includes less developed countries that tend to have lower cancer incidence, therefore pulling down the worldwide incidence. The reason behind it lies most likely in lower surveillance and poorer detection methods, although a difference in risk factor distribution cannot be ruled out [29].

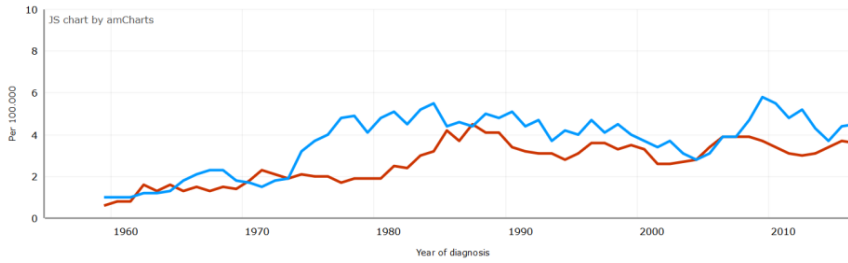

Figure 2 Age-standardized incidence per 100.000 of multiple myeloma in Iceland from 1955-2016 [30]

1.1.2 Survival

In the past decade survival rates have improved drastically. Based on the Surveillance, Epidemiology, and End Results (SEER) data, 5-year relative survival rates in multiple myeloma improved from 27% for the 1987-1989 period to 49% for the 2005-2011 period [31]. A European multi-center study found that relative survival increased from 30% to 40% between 1997-1999 and 2006-2008 [32]. Additionally, results from a study on the Swedish population, including 21,502 multiple myeloma patients over 40-year period confirms these results [33]. The most likely explanation for this increase in survival is changes in treatments with introduction of major therapeutic agents and procedures. In the recent decade, the effect of these new agents explains possibly most of the increase in survival, as they were introduced in 1999-2000 [28, 34]. In the subsequent years, they were used frequently as first-line treatment of multiple myeloma [33].

1.1.3 Risk factors

Multiple myeloma is a multifactorial disease with variety of risk factors that span numerous life events, some with many controversies. The most established risk factors include age, sex, racial disparities, and personal or family history of related diseases [5, 6, 12-14, 25, 29]. The prevalence of multiple myeloma increases with age [25]. The median age of patients at diagnosis is approximately 70 years with 37% of patients being younger than 65 years [25], and studies have shown that men are at 1.5 times higher risk of being affected than women [5, 29].

Striking differences in incidence of multiple myeloma between races has been observed [6, 35]. Waxman et al. evaluated the difference in incidence of multiple myeloma among African and European Americans in a cohort of 35.000 myeloma patients, using the SEER registries. They found the incidence of multiple myeloma to be 11 per 100,000 and 4.9 per 100,000 in African and

European Americans, respectively [6], suggesting underlying biological or socio-demographical differences among the two races.

Several studies have reported an increased risk of developing multiple myeloma among persons with a first-degree family history of multiple myeloma [12-14]. In a review of the epidemiological literature of multiple myeloma by Alexander et al. from 2007 it was reported that the approximate range of observed associations was 1.5-5.0 in positive family history of multiple myeloma or related diseases in first degree relatives [12]. These results were confirmed in a population-based study from 2009 on 37,838 first degree relatives of 13,896 patients with multiple myeloma in Sweden [13] and in a pooled analysis performed by the International Multiple Myeloma Consortium [14].

Several lifestyle factors have been analyzed in association with multiple myeloma. Overweight and obesity have consistently been found to increase the risk of multiple myeloma [10, 36-40], but, physical activity and smoking on the other hand have not been found to be associated with multiple myeloma [41, 42]. Furthermore, there are indications that certain dietary factors might be associated with multiple myeloma, however, the literature is scarce and inconclusive [17-23].

A systematic review and meta-analysis from 2015 reported results on occupational risk factors for multiple myeloma [43]. Several occupations seem to be associated with increased risk of multiple myeloma, mostly occupations where exposure to pesticides (farmers), metal and minerals (fire fighters), and other chemicals (hairdressers and painters) is high [43]. Additionally, the herbicide Agent Orange, used in warfare in the Vietnam War, has been found to increase risk of four B-cell lymphoid malignant neoplasms, including multiple myeloma [44], most likely through increased risk of MGUS [45, 46].

Other, less established, risk factors include presence of other diseases, such as infectious, metabolic, and autoimmune diseases, although with inconclusive results [43, 47], and genetic studies have reported findings on promoter methylation in the P15INK4b and P16INK4a genes and increased risk of multiple myeloma [48].

1.2 Monoclonal gammopathy of undetermined significance

All cases of multiple myeloma are preceded by MGUS, a premalignant asymptomatic condition characterized by the presence of an M-protein in serum without evidence of multiple myeloma or other lymphoproliferative diseases [8, 24]. Smoldering multiple myeloma is an intermediate stage between MGUS and active multiple myeloma, but is distinguished from MGUS by a much higher risk of progression to multiple myeloma [2, 24]. MGUS, smoldering multiple myeloma, and multiple myeloma are therefore a spectrum of the same disease process.

MGUS is the most common plasma cell disorder [2] and the term was first introduced over 40 years ago, when it was already realized that patients with benign monoclonal gammopathy were at risk of developing symptomatic multiple myeloma, Waldenström's macroglobulinemia, or other related disorders during long-term follow-up [49-51]. MGUS is asymptomatic and is usually discovered by chance during work-up of other clinical disorders and symptoms [51]. It is defined as by an elevated M-protein concentration (<30 g/L), fewer than 10% monoclonal plasma cells in the bone marrow, and absence of hypercalcemia, renal insufficiency, anemia, or bone lesions, and no evidence of other B-cell proliferative diseases [52, 53].

MGUS is commonly divided into 3 groups; Non-IgM MGUS, IgM MGUS, and light chain MGUS (LC-MGUS), based on the symptomatic diseases these precursor conditions progress to. Non-IgM MGUS is a precursor condition of multiple myeloma, IgM MGUS of Waldenström's macroglobulinemia, and in rare cases IgM multiple myeloma, and LC-MGUS of light chain multiple myeloma and amyloidosis [24]. LC-MGUS is defined as the presence of an abnormal ratio between the FLC´s (kappa and lambda), and increased concentration of the involved light chain, no sign of monoclonal immunoglobulin heavy chain, fewer than 10% monoclonal plasma cells in the bone marrow, and absence of end-organ damage [54].

1.2.1 Prevalence

The prevalence of MGUS increases with age and is reported to be found in approximately 5-10% of those older than 70 years (Figure 3) [11]. For LC-MGUS the prevalence has been estimated to be between 0.7% and 0.8% [54, 55]. In a population-based study conducted in Olmsted County, Minnesota, MGUS was assessed in all residents (N=21,463) 50 years and older. The prevalence of MGUS was found to be 3.2% in the total population, 5.3% in those older than 70 years and was up to 7.5% in those older than 85 years [11].

1.2.2 Progression and survival

The rate of progression from MGUS to multiple myeloma and related diseases ranges from 0.3-1.5% per year, depending on MGUS subtype [24]. Several factors influence the rate of progression, such as concentration of the M-protein, type of M-protein, and presence of a pathological FLC ratio [56-60]. The progression rate of non-IgM MGUS is 1% per year [52], for IgM MGUS it is 1.5% [53], and 0.3% for LC-MGUS [54, 61]. This low risk of progression indicates that the majority of MGUS patients will never develop a lymphoproliferative malignancy during the life course and most likely die of other causes. Since MGUS is, by definition, asymptomatic and is most often diagnosed by chance,

data on survival in MGUS is limited. The studies that have examined survival, point towards inferior survival in MGUS when compared to the general population or matched controls [57, 62, 63], and the causes of death span a wide range of diseases, in most cases diseases that led up to the detection of MGUS [57, 63].

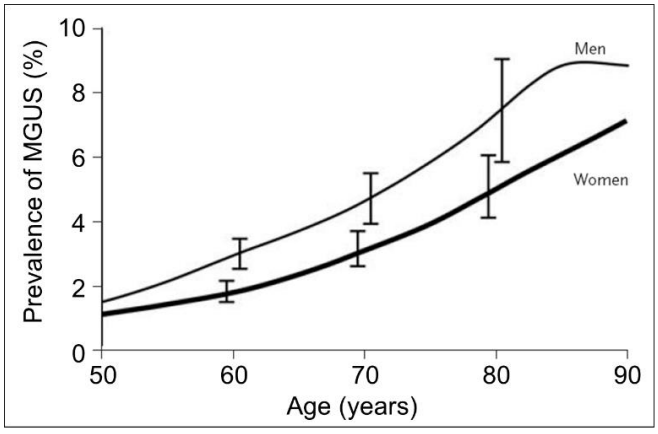

Figure 3 Prevalence of MGUS according to age

The I bars represent 95% confidence intervals. Years of age greater than 90 years has been collapsed to 90 years of age. Reproduced with permission from [11], copyright Massachusetts Medical Society.

1.2.3 Risk factors

MGUS and multiple myeloma are within the spectrum of the same disease with similar established risk factors; age, sex, race, and family history. As previously mentioned MGUS is highly dependent on age [11], and its prevalence increases rapidly with increasing age (Figure 3). Studies have reported a higher risk of MGUS in males, with age-adjusted rates of 4.0% for men and 2.7% for women in the Olmsted county population [11]. Researchers have also found the prevalence of MGUS to be approximately twice as high in individuals of African descent when compared to other races, suggesting a genetic predisposition, although a contributing effect of socioeconomic status cannot be ruled out [64, 65]. Studies have also suggested the increased risk of multiple myeloma in individuals of African descent may be a result of in increased risk of MGUS, as the rate of progression from MGUS to multiple myeloma is similar between races [35].

Additionally, first-degree relatives of people with MGUS are at increased risk of developing MGUS and other related diseases [66, 67], suggesting a presence of shared underlying genetic factors, shared environmental influences, or

interaction between both. Other possible risk factors include obesity, smoking, chemical exposure, and presence of other diseases, such as hypertension, diabetes, coronary artery diseases, and autoimmune diseases [45, 47, 68, 69].

1.3 Obesity and diet and monoclonal gammopathies

1.3.1 Obesity

In 2014, it was estimated that 640 million adults and 110 million children and adolescents were obese, and the estimated age-standardized prevalence of obesity was 14.9% among women, 10.8% among men, and 5.0% among children [10, 70]. Overweight and obesity have consistently been recognized as risk factors for many common cancers [71-73], such as cancers of the colon [73, 74], gastric cardia [75], liver [76], gallbladder [77], pancreas [78], kidney [79], and for esophageal adenocarcinoma [80]. On the basis of recent estimates, the obesity-related cancer burden among men and women in North America, Europe, and the Middle East represents up to 9% of the total cancer burden [10, 81].

Recently, the WHO concluded that there is sufficient evidence for the association between weight and 13 types of cancers, including multiple myeloma (Figure 4) [10]. Several large scale studies and meta-analyses have reported a positive association between BMI and multiple myeloma [36-40]. A meta-analysis of prospective studies from 2011 reported that a 5 kg/m^2 increase in BMI was associated with 12% increased risk of multiple myeloma incidence [40]. A recent large scale prospective study on 575 incident multiple myeloma cases over five million person-years of follow up, examined the association between BMI through adulthood and risk of multiple myeloma [39]. The risk of multiple myeloma increased 17% per 5 kg/m^2 increase in cumulative average BMI and 28% per 5 kg/m^2 increase in young adulthood BMI. These studies indicate that BMI both in early and later adulthood are risk factors for multiple myeloma and that obesity may be the first recognized modifiable risk factor for multiple myeloma.

Data on the association between obesity and MGUS is limited, and results inconclusive [65, 68, 69]. A study (N = 1,996) on 60 women with MGUS reported a significant association between obesity and MGUS [69]. However, a study (N = 12,482) on 365 MGUS patients did not find an association, although the prevalence tended to be higher with increasing BMI [65]. A recent study by Boursi et al. aimed to evaluate metabolic risk factors for MGUS and found an association between obesity, diabetes, and hypertension and MGUS. However, after adjusting for number of lab tests performed prior to index date, the association for all these factors attenuated, suggesting a detection bias in previously reported metabolic risk factors, since the probability of detecting an asymptomatic condition, like MGUS, is higher among individuals with medical comorbidities [68].

Figure 4 Releative risk for the effect of obesity on 13 types of cancer

The effect of obesity on progression from MGUS to multiple myeloma has recently gained attention. In 2016, Veld et al. assessed abdominal adipose tissue parameters in patients with MGUS (n = 40) and multiple myeloma (n = 32) [82]. They found that patients with multiple myeloma had higher abdominal fat and higher fat metabolic activity compared with patients with MGUS, indicating that these parameters may serve as biomarkers of progression from MGUS to multiple myeloma. Chang et al. recently investigated the role of obesity in the progression of MGUS to multiple myeloma, using the US Veterans Health Administration database [83]. They identified 7,878 MGUS patients, of which 329 progressed to multiple myeloma, over a median follow-up of 68 months. They found that both overweight (BMI 25.0-29.9 kg/m^2) and obesity (BMI ≥ 30 kg/m^2) were associated with an increased risk of progressing from MGUS to multiple myeloma. Taken together, previous studies suggest that obesity plays a role in myelomagenesis, most likely by increasing the risk of progressing from MGUS, rather than increasing MGUS risk.

The mechanisms through which obesity might influence multiple myeloma, and possibly MGUS, are not yet established. However, there is a strong evidence that physiological dysfunction of adipose tissue in individuals with obesity can promote multiple myeloma pathogenesis [84]. Adipokines are polypeptide hormones with pro- and anti-inflammatory properties and are secreted by the adipose tissue. Adiponectin, the most abundant adipokine, is negatively associated with obesity as circulating levels are lower with increasing obesity [85-87]. It has been suggested that adiponectin may prevent development of multiple myeloma by suppressing production of pro-inflammatory cytokines while inducing other anti-inflammatory cytokines. Thereby, affecting the environment where myeloma cells are able to thrive by altering pathways that are associated with survival and proliferation of malignant plasma cells [88-91]. Hofmann et al. tested, in a prospective study, the association between circulating adiponectin levels and multiple myeloma risk and found a clear inverse relationship between circulating adiponectin and myeloma risk. Additionally, when adjusting for adiponectin in the association between BMI and multiple myeloma the association was largely attenuated [92].

1.3.2 Diet

Based on current knowledge, it is believed that factors related to energy balance might account for 10-15% of the cancer burden in the United States, including dietary factors [93], indicating that by modifying the diet, a good fraction of cancer incidence can be prevented. The literature regarding the role of diet in the development of multiple myeloma is scarce and inconclusive, with the strongest evidence existing for an inverse association with intake of fish. A meta-analysis by Caini et al. from 2016 [18] on the association between foods of animal origin and multiple myeloma indicated an inverse association between fish and risk of multiple myeloma. That is consistent with a 2015 meta-analysis by Wang et al. [94] that concluded that the highest versus lowest category of fish consumption was inversely associated with risk of multiple myeloma. Additionally, suggesting a nonlinear dose-response relationship. Although the heterogeneity was high in both meta-analyses, the consistency of the preventive effects of fish consumption adds to the reliability of the results. With regards to other foods of animal origin Caini et al. reported a non-significant increase in risk for red meat and processed meat but no association for white meat, cheese, and milk [18]. Although not significant, the results reinforce the indication of carcinogenic effect of red and processed meat, as previously suggested [16], and suggest replacing its intake with consumption of fish.

Several studies have examined the association between consumption of fruit and vegetables and risk of multiple myeloma, with inconclusive results. Few case-control studies have reported an inverse association with total vegetable

consumption [95], green vegetables [22], cruciferous vegetables [17, 20], and tomatoes [20] while reporting no association for other subtypes of vegetables. However, a recent systematic review and meta-analysis of prospective studies concluded, based on three eligible studies, that the risk of vegetable consumption on multiple myeloma pointed towards a null association, also for vegetable subgroups [21]. Few case-control studies have examined the association between fruit intake and risk of multiple myeloma, reporting no association [17, 20, 22]. That is consistent with Sergentanis et al. that reported no association for fruits or subtypes of fruit in a systematic review and meta-analysis of prospective studies [21].

Chatenoud et al. analyzed the association between whole grain intake and multiple types of cancer in a case-control study and found, based on 120 cases of multiple myeloma, that the highest tertile of intake was not associated with risk of multiple myeloma when compared to the lowest tertile of intake. But, when analyzing the association stratified by gender, the association was significant for women but not for men [19].

Taken together, the current literature on the association between diet and multiple myeloma is limited and inconclusive for most types of food, therefore the role of diet in multiple myeloma is unclear. Current studies indicate an inverse association with intake of fish and positive association with intake of red and processed meat. No studies to date have analyzed the role of diet in MGUS or on MGUS progression.

The identified inverse association between fish intake and risk of multiple myeloma does have a biological plausibility. Certain types of fish are rich in Omega-3 essential fatty acids, including eicosapentaenoic acid and docosahexaenoic acid, which have been reported to inhibit the growth of myeloma cells [94, 96], and vitamin D is believed to have an effect on cell cycle arrest and regulation of cellular apoptosis, and therefore inhibiting myeloma cell growth [94, 97, 98].

The possible mechanism linking red and processed meat and multiple myeloma is unclear, and the mechanistic evidence is mainly available for cancers of the digestive tract, where the strongest association with red and processed meat has been found [16]. The evidence suggest an effect of oxidative stress and genotoxicity caused by multiple meat components and meat processing and cooking, such as haeme iron, N-nitroso-compounds, and heterocyclic and polycyclic aromatic hydrocarbons [16].

1.3.3 Dietary pattern

Diet is a complex exposure variable. Most studies to date use foods or nutrient intake as exposure when analyzing the association between diet and different health outcomes, although, in recent decades a greater emphasis has been put

on the use of dietary patterns as an alternative and complementary approach [99, 100]. Using dietary patterns to represent combined effects of foods or nutrients consumed in a person's diet addresses the challenge of differentiating between correlated foods or nutrients that confound each other [99].

Statistical methods are usually used to derive dietary patterns from collected dietary data. Four methods have been described in the literature, both theoretical and empirical driven methods [99-101]. A priori indices is a theoretical driven method that is usually based on interpretation of the literature relating diet to health, and the patterns are constructed from dietary recommendation or guidelines [99, 100]. An example is the Healthy Eating Index that was created to measure adherence to the Dietary Guidelines in the United States [102] and the Mediterranean dietary index first described in a survival study of Greek women [103]. Factor analysis, cluster analysis, and reduced rank regression are examples of empirically driven methods. Factor analysis uses standard multivariate statistical method, most commonly principal component analysis (PCA), to define dietary patterns using dietary information usually collected from food frequency questionnaires (FFQ) or dietary records. PCA aggregates food items or food groups based on which foods tend to be consumed or avoided by the same person. Each individual in the dataset gets a score for each pattern derived based on adherence to the pattern, each pattern can then be used as a continuous exposure variable when analyzing the association with the outcome of interest [99, 100]. Cluster analysis also uses multivariate statistical methods but is different from factor analysis as it creates clusters of individuals with relatively similar diets and creates a single categorical exposure variable. The clusters need to be analyzed by comparing dietary profiles across clusters for interpretation of dietary patterns [99, 100]. Reduced rank regression is the most recent method used in nutritional epidemiology [104]. It is similar to that of factor analysis as it uses multivariate statistical model to create a number of continuous factors but is different in the sense that it connects one set of variables, such as dietary data, with another set of variables, such as a set of nutrients or biomarkers that can be linked to a disease of interest. By doing so, this method offers a biological basis for the dietary pattern, and provides a stronger evidence for a causal effect of the pattern than the factor analysis does [99].

To date, no studies using dietary patterns as exposure, have been conducted on the association between diet and multiple myeloma and only one on other hematologic cancers [105]. However, this method has been used in studies on other types of cancer, such as breast, prostate, colorectal, and gastric cancer [106-110]. The most common method used is the a priori indices to identify adherence to the Mediterranean dietary pattern and factor analysis (PCA). Studies on western populations most often identify two patterns when using factor analysis, often described as the Prudent or Healthy dietary pattern and

Western dietary pattern [99]. The first pattern is usually characterized by greater intake of fruit and vegetables, whole grains, and fish, and the second by greater intake of red and processed meat, high fat dairy, potatoes, and white bread [99]. Although the patterns are not identical between studies on different population, results are consistent in describing a favorable role of the Prudent/Healthy pattern and unfavorable role of the Western pattern [106, 108, 109, 111].

Although valuable, studying single or few foods or nutrients has several limitations, both theoretical and methodological. A review from 2002 discussed the advantages and disadvantages of using dietary patterns as exposures in dietary studies and the limitations associated with single food or nutrient studies [100]. The major limitations are that some types of foods are highly correlated, making it difficult to examine their effects separately. People do not eat isolated foods or nutrients, but meals that contain variety of foods with complex nutrient combinations that interact and sometimes the effect of the food or nutrient is so small that it is not detected, but can be detected as a cumulative effect of multiple foods or nutrients in a pattern [100]. Using dietary patterns offers a different approach and addresses most of these limitations as they describe the way foods are actually combined in free-living people [99].

Studying diet as an exposure is complex and calls for additional approaches, such as dietary patterns. However, dietary pattern analysis cannot replace single food or nutrient analysis but can serves as complementary approach when examining the association between diet and diseases.

1.4 Study motivation

There is a gap in the literature with regards to modifiable risk factors for MGUS and multiple myeloma. In recent years, obesity has been described as a risk factor of multiple myeloma, but whether it is due to increased risk of MGUS or if it affects MGUS progression or both remains unclear. Studies on diet and risk of multiple myeloma are limited and inconsistent and to date no studies have been conducted on risk on MGUS or MGUS progression. The Icelandic Heart Association (IHA) started a population-based cohort study (the Reykjavik Study) in 1967 with the aim of examining risk factors for cardiovascular diseases. All men and women residing in the Reykjavik metropolitan area in 1966 and born during the years of 1907-1935 were invited to participate (N = 30,795). In 2002, the IHA initiated a continuation of the Reykjavik Study called the Age, Gene/Environment Susceptibility-Reykjavik Study (AGES-Reykjavik Study). The AGES-Reykjavik Study participants had extensive examination, such as anthropometric assessments, computed tomography (CT), bioelectrical impedance analysis, blood draw, and they answered a detailed lifestyle related questionnaire including an FFQ on dietary habits across the lifespan. The IHA

data offered the possibility to screen the whole AGES-Reykjavik Study cohort for MGUS and it provided a solid base to study the association between lifestyle related factors such as diet and obesity and MGUS. Furthermore, by linking the AGES-Reykjavik Study data to the nationwide cancer registry opened the opportunity to additionally analyze the association between obesity and diet and progression from MGUS to multiple myeloma and other related diseases.

2 Aims

The overarched aim of this thesis was to explore the associations between obesity and diet across the life course and MGUS and LC-MGUS using data from the population-based AGES-Reykjavik Study. Our aim was also to analyze whether these factors were associated with progression from MGUS or LC-MGUS to multiple myeloma and other lymphoproliferative diseases.

2.1 Study I

The aim of study I was to analyze the association between 11 different obesity assessments and MGUS and LC-MGUS. Secondly, our aim was to analyze whether the same assessments were risk factors for progression from MGUS or LC-MGUS to multiple myeloma and other lymphoproliferative diseases.

2.2 Study II

The aim of study II was to analyze the association between diet throughout the lifespan and MGUS and LC-MGUS. Secondly, our aim was to analyze whether diet was a risk factor for progression from MGUS or LC-MGUS to multiple myeloma and other lymphoproliferative diseases.

2.3 Study III

The aim of study III was to analyze whether adherence to empirically derived dietary patterns from the adolescent period (14-19 years) was associated with risk of MGUS or LC-MGUS later in life.

3 Materials and methods

3.1 Study population

The AGES-Reykjavik Study is a continuation of the prospective population-based Reykjavik Study [112-114]. The Reykjavik Study originally comprised a sample of 30,795 men and women (mean age 52.1 years and 53.3 years, respectively) residing in the Reykjavik metropolitan area. The study was initiated in 1967, when all residents, born between 1907 and 1935 were invited to participate in the study with the objective of examining risk factors for cardiovascular diseases. Approximately 19,000 of the invited participants consented to participate during the years 1967-1996 [112-114]. The AGES-Reykjavik Study was initiated in March 2002. One of its main objectives was to explore how genetic, behavioral, and environmental risk factors are associated with complex traits and diseases that manifest late in life. Of the 11,549 Reykjavik Study cohort members still alive, 8,030 individuals were randomly chosen to take part in the study. By February 2006, when the study ended, 5,764 (71.8%) had participated [112].

3.2 AGES-Reykjavik Study data collection

The AGES-Reykjavik Study examinations were performed during three clinic visits within 4- to 6-week time window [112]. The first clinic visit included a blood pressure measurement, blood draw, electrocardiography, anthropometry, measures of physical and cognitive function, and an extensive questionnaire including questions on health history, lifestyle, medication history, and an FFQ from three different periods; adolescence, midlife, and late life. The second examination day included imaging protocols using magnetic resonance imaging, CT scans, and ultrasound instrumentation, and the third day included vision screening, a hearing test, and dementia assessment [112].

3.2.1 Obesity assessment – Study I

Physique was assessed during the first clinic visit and for this paper, 11 different measures of obesity were included. From the AGES-Reykjavik Study baseline, we included weight (kg), height (m), BMI (kg/m^2), percent body fat (%), fat (kg), total body fat (cm^2), visceral fat (cm^2), subcutaneous fat (cm^2), and two separate assessments of abdominal circumference (cm). For this study self-reported lifetime maximum weight (assessed at AGES-Reykjavik Study baseline) and measured BMI (kg/m^2) from midlife, obtained from the Reykjavik Study data, was

additionally included. Weight and height were measured multiple times and a mean number found. Percent body fat and fat in kg were calculated from bioelectric impedance (BIA). CT imaging of the abdomen at the level of L4/L5 vertebrae was performed to assess visceral fat area, subcutaneous fat area, total body fat area, and abdominal circumference [112]. Abdominal circumference was additionally measured multiple times in a conventional way [115] and a mean number found. Midlife BMI was calculated from weight and height measured at Reykjavik Study entry.

BMI is the most commonly used method to assess obesity in epidemiological studies as it is relatively easy to measure in large populations [116]. However, BMI is often criticized as it does not distinguish between fat and lean mass and it is often discussed that it should be either substituted or supplemented with other more precise measures of body composition and fat distribution. Nevertheless, studies have shown that BMI highly correlates with other measures such as waist circumference, skinfold measures, BIA, and CT [116-118], suggesting that BMI can be just as good at predicting some outcomes [116, 117]. BIA is also an accessible and cost-efficient method to assess obesity, but offers a more detailed information on body composition than the BMI does, and correlates well with waist circumference [119, 120]. It estimates the whole body fat content but cannot distinguish the fat distribution; visceral fat or subcutaneous fat. However, it has been shown to be somewhat correlated with imaging techniques such as CT [118, 121, 122]. CT measures offer greater precision for analysis of fat distribution, although at greater financial cost, and it is currently the gold standard for assessment of intra-abdominal adipose tissue. [116, 118, 123]. Although highly correlated with BMI, studies that need more accuracy and precision in fat distribution, such as studies on cardiovascular and metabolic outcomes, benefit greatly from CT measures [118]. For the most precise and accurate assessment of the effect of obesity on MGUS and progression all the above-mentioned measures were used for study I.

3.2.2 Dietary assessment – Study II and III

At AGES-Reykjavik Study entry, the participants provided retrospective information on dietary habits during adolescence (14-19 years old) and midlife (40-50 years old), as well as information on current dietary habits using an FFQ. The FFQ included a total of 63 questions, 16 from the adolescent period, 17 from the midlife period, and 30 from the late life period. The questions represented common food and food groups from these periods such as fish (total fish intake, fish in salad or as topping, and salted or smoked fish intake), fish oil, meat (total meat intake and salted or smoked meat), milk and milk products, fruit, vegetables, rye bread and flatbread, blood or liver sausage, oatmeal and muesli, potatoes, and whole wheat bread. There were additional questions on for

example cake, cookies, pastry, and candy in the late life questionnaire, but in the current studies we only used foods and food groups that were included in the questionnaire from all three life stages, except for whole wheat bread (midlife and late life only) as it was relatively uncommon in Iceland until the middle of the 20th century [124].

The participants reported frequency of intake during each time period using the following response categories for fish, fish in salad or as topping on bread, meat, milk and milk products, fruit, vegetables, rye bread and flatbread, blood or liver sausage, oatmeal and muesli, potatoes, and whole wheat bread: *never*, *less than once a week*, *1-2 times a week*, *3-4 times a week*, *5-6 times a week*, *daily,* and *more than once a day*. For fish oil the categories were the same except for the last option: *more than once a day*. The categories for salted or smoked meat and salted or smoked fish were: *never*, *less than once a month*, *1-3 times a month*, *1-2 times a week*, *3-6 times a week*, and *daily or more often*.

The FFQ included three questions on fish consumption; fish as a main meal (including salted or smoked fish), fish in salad or as topping on bread, and salted or smoked fish. Weekly intake of fish as a main meal and fish in salad or as topping on bread was converted into total fish portions per week. The weekly intake of these foods was converted to daily intake. *Never* was converted to zero per day, *less than once a week* to 0.07 per day, *1-2 times per week* to 0.21 per day, *3-4 times per week* to 0.5 per day, *5-6 times per week* to 0.79 per day, *daily* to once per day, and *more than once a day* to 1.5 per day. Based on an average portion in a nationwide survey [125] a standard portion of fish was estimated to be 150 g and for fish in salad or as topping on bread it was 40 g, the number of portions of fish in salad or as topping of a fish meal was therefore calculated as 0.27 (40/150). The daily estimate of fish in salad or as topping on bread was therefore multiplied with 0.27 and computed with the daily estimated intake of fish meals. The total outcome was multiplied with seven to get total portions per week [126].

Assessing the validity of a questionnaire is essential before studying the relationship between diet and health-related outcomes. The AGES FFQ has been validated for midlife and late life periods [127, 128], whereas the adolescent intake cannot be directly validated as there are no available data on individual food intake from this period in the participant's lives. However, there is a household survey on average intake from 1939 that shows similar distribution of intake according to residence in rural and coastal fishing areas as found in the present study [129, 130].

For validation of midlife diet, dietary data from the 1990 Icelandic National Dietary Survey was used. A total of 174 survey participants who completed the midlife AGES FFQ had also completed the national survey in 1990 and the validity was then assessed by comparing answers of the FFQ to dietary data

from the national survey [127]. For men, all questions were found acceptable ($p \leq 0.05$) to rank individuals according to intake ($r = 0.26$-0.53) except fruit ($r = 0.18$), rye bread and flat bread ($r = 0.15$), and vegetables ($r = 0.15$). For women, all questions, except question on rye bread and flat bread ($r = 0.07$), whole wheat bread ($r = 0.05$), and vegetables ($r = 0.08$), were found acceptable to rank individuals according to intake ($r = 0.21$-0.56). Validation of intake of fish in a salad or as topping on bread, salted or smoked meat, and salted or smoked fish could not be assessed since as information from the two methods could not be compared [127].

For current dietary habits a subsample ($n = 128$) of participants in a study focusing on the effects of training and food supplements on various health-related factors was used [131]. The subjects were healthy individuals aged 65 years and older. Participants answered the AGES FFQ and subsequently filled out a 3-day weighed food record within 2 weeks. The validity was assessed by comparing the answers of the FFQ to the weighed food records [128]. For men, questions on potatoes, fruit, vegetables, oatmeal and muesli, milk, and fish oil were found suitable to rank individuals according to intake ($r = 0.33$-0.51) but questions on meat meal, fish meal, fish in salad or as topping on bread, blood or liver sausage, rye bread and flat bread, and whole wheat bread were not found to be correlated to the reference method ($r = 0.05$-0.23). For women, all questions were found acceptable to rank individuals according to intake ($r = 0.28$-0.48), except for meat ($r = 0.11$), fish meal ($r = -0.02$), and potatoes ($r = 0.01$). The validity of intake of salted or smoked meat and salted or smoked fish could not be assessed since methods could not be compared [128].

3.2.3 Extraction of dietary patterns – Study III

For study III, PCA was used to extract dietary patterns from the adolescent FFQ. This empirically derived method identifies dietary variables (patterns), based on foods that tend to be commonly consumed (or avoided) together by the same person [99, 132]. New linear factors (patterns) are made, and a score for each pattern for every person in the dataset is calculated by assigning weights to their frequency of use of each food [99]. The score calculated for each person indicates his or her adherence to the pattern, and the higher the adherence, the more closely the person´s diet conforms to that particular pattern. The correlation coefficients defining the dietary pattern are called factor loadings and represent the correlation between each input variable (foods or food groups) and the extracted dietary pattern. A positive factor loading indicates that the corresponding foods or food groups are positively correlated with the dietary pattern, whereas negative factor loadings indicate an inverse correlation. The number of patterns generated in a dataset can vary. The first pattern explains the greatest between-person variation and then the variation diminishes rapidly in the

next patterns. Usually only two or three patterns provide groupings that have intuitive meaning [99].

For our study the number of patterns to retain was based on interpretability of the eigenvalues and the scree plot. The eigenvalues represent the amount of variation each pattern accounts for and if a pattern has a low eigenvalue, then it contributes little to the explanation of variances and has little intuitive meaning. The scree plot plots the eigenvalues for each pattern, and often one or more breaks in the plots are identified and used as indicator for how many patterns should be retained.

A total of 13 patterns emerged. Four patterns had an eigenvalue greater than one; the first had an eigenvalue of 2.5, the second had 1.8, the third had 1.15, and the fourth had 1.09. A clear break in the scree plot was noticed after the second pattern and therefore the first two patterns were retained. Table 1 shows the two extracted patterns, including the factor loading coefficient between the foods and the extracted pattern. The first pattern was characterized by high consumption of salted or smoked meat, salted or smoked fish, blood or liver sausage, rye bread, milk and milk products, oatmeal, and potatoes. This type of diet was common during the early 20th century, particularly in the rural areas of Iceland, therefore the pattern was labelled “Traditional early 20th century diet”. The second pattern was characterized by high intake of fruit, vegetables, and fish on bread or in salad and was thus labelled “Healthy diet” (Table 1).

Table 1 Factor loading coefficients between the food groups and the extracted patterns

Dietary pattern	Food	Factor loading coefficient	Cumulative variance explained (%)
Traditional early 20th century diet	Salted/smoked meat	0.78	19
	Salted/smoked fish	0.73	
	Blood sausage/liver sausage	0.59	
	Rye bread	0.47	
	Milk & milk products	0.42	
	Oatmeal	0.35	
	Potatoes	0.30	
Healthy diet	Fruit	0.77	33
	Vegetables	0.76	
	Fish on bread or in salad	0.64	

Factor loadings are correlation coefficients between input variables (foods) and the extracted patterns. Food groups are sorted by size of loading coefficients. For simplicity food groups with factor loadings between 0.30 and -0.30 are not listed.

3.3 Outcome ascertainment and follow-up

MGUS cases were identified during the years of 2013-2014 using a conventional agarose-gel SPEP. A 0.5 mL serum sample, collected at AGES-Reykjavik Study entry (2002-2006), was shipped to the Multiple Myeloma Research Laboratory at

the National Cancer Institute in the United States, where protein assays were performed. Samples with an equivocal or definite M-protein present on SPEP were then subjected to serum protein immunofixation for confirmation and typing of the M-protein [11]. MGUS cases were defined as having M-protein bands visible on SPEP and an M-protein concentration below 30 g/L [52]. To identify LC-MGUS cases, serum FLC assay was performed on all samples [59]. The criteria for LC-MGUS was having no M-protein band visible on SPEP, a pathological FLC ratio (<0.26 or >1.65) in combination with an increased concentration of the light chain involved (f-kappa >19.4 mg/L and f-lambda >26.3 mg/L) [133].

Cases of multiple myeloma and other lymphoproliferative diseases (Hodgkin's and non-Hodgkin's lymphoma, Waldenström's macroglobulinemia, lymphoid leukemia, chronic lymphocytic leukemia, and acute lymphoblastic leukemia) were found through linkage with the nationwide Icelandic Cancer Registry. The registry has very high diagnostic accuracy and completeness (99%) and is in accordance with internationally accepted standards [134]. The participants were followed from AGES-Reykjavik Study entry date (March 2002-February 2006) to March 2014.

3.4 Other covariates

Information on potential confounders were retrieved both from the AGES-Reykjavik Study and the Reykjavik Study. From the AGES-Reykjavik Study we retrieved information on age, sex, lifetime (20-65 years old) physical activity (never/rarely/occasionally, moderate/high), and education (primary/secondary, college, university). Information on midlife BMI and early life residency (capital area, sea-side village, rural area, combination of sea-side village and rural area) was retrieved from the Reykjavik Study. Classification of early life residency has been previously published [130].

3.5 Exclusion from analysis

A total of 5,764 individuals were enrolled in the AGES-Reykjavik Study during the years of 2002-2006. We excluded 40 (0.7%) individuals from analysis in our study, 21 due to previous lymphoproliferative diseases, 16 due to missing blood sample, one had a missing consent form, and one had an M-protein concentration above the limit for MGUS and therefore fulfilling the criteria for smoldering multiple myeloma. One individual was excluded from the progression analysis in paper I and II due to zero follow-up days.

3.6 Statistical analysis

3.6.1 Study I

Logistic regression was used to estimate the association between the 11 obesity markers and MGUS and LC-MGUS and the results presented as odds ratios (OR) with 95% confidence interval (95% CI). Analysis was performed for both MGUS and LC-MGUS and adjustment was made for age and sex in a multivariable analysis. We additionally adjusted for height in models were height is not considered in the exposure variable, such as weight, waist circumference, and CT measures. As height did not affect the estimates it was not included in the final models.

The association between history of obesity from midlife to late life (AGES-Reykjavik Study entry) and MGUS and LC-MGUS was additionally tested by cross-classifying individuals according to their BMI status from these two time points. Individuals with low midlife BMI (<25 kg/m^2) and low BMI in late life were the reference group for increase in BMI, decrease in BMI, and high BMI (≥ 30kg/m^2) at both these time points. Logistic regression, using the cross-classification of BMI as exposure, was performed, adjusting for age and sex.

Cox proportional-hazard regression was used to test whether the obesity markers were risk factors for progression from MGUS or LC-MGUS to multiple myeloma and other lymphoproliferative diseases and results presented as hazard ratios (HR) with 95% CI's. Adjustment was made for age and sex.

All statistical analysis were performed in R version 3.1.2 [135].

3.6.2 Study II

We used logistic regression to test the association between 12 foods or food groups from the adolescent period and 13 from the midlife period and MGUS and LC-MGUS. Results are presented as OR's with 95% CI's. For each type of food or food group, participants were grouped according to their frequency of intake or portions per week (total fish consumption only). Each type of food or food group was analyzed in an age and sex adjusted model (Model 1) and additionally in a fully adjusted model where all the foods and food groups were simultaneously added to the model, including age and sex (Model 2). An additional adjustment was made for physical activity and midlife BMI, but since the adjustment did not affect our results, they were not included in the final models of the study.

We additionally cross-classified the adolescent and midlife intake by combining individuals with low intake at both time points and used them as a reference group for increase in intake, decrease in intake, and constant high intake during these two time points. We then used logistic regression to test the association between the cross-classified categories of intake and MGUS and LC-MGUS combined. In these models we adjusted for age and sex.

Cox proportional hazard regression was used to test whether late life diet was a risk factor for progression from MGUS or LC-MGUS to multiple myeloma or other lymphoproliferative diseases. Due to few cases of multiple myeloma, we analyzed MGUS and LC-MGUS together. Results are presented as HR's with 95% CI's. All models were adjusted for sex and age. We further adjusted our models for BMI and physical activity and we tested the association in a model where all the foods were simultaneously added to one model. Due to few numbers of cases and therefore low statistical power, and no effect on results, these analyses were not included in the study. All analysis were performed in R version 3.3.3 [135].

3.6.3 Study III

PCA was used to identify dietary patterns. Eigenvalues and scree plot were used to determine how many dietary patterns to extract, as described in chapter 3.2.3. After creating linear variables for the extracted dietary patterns, that represent each subjects' adherence to each pattern, we divided the patterns into tertiles. Logistic regression was used to test the association between the mid and highest tertile of adherence and MGUS and LC-MGUS, using the lowest tertile as a reference. We also tested for linear trends by converting the tertiles of adherence into an ordered variable. The results are presented as OR's with 95% CI's and p-value for trend. Adjustment was made for age, sex, lifetime physical activity (20-65 years), education, residency, and midlife BMI.

All analysis were performed in R version 3.4.0 [135].

3.7 Sensitivity analysis

We found the prevalence of LC-MGUS to be substantially higher in our cohort (4.8%) than previously reported (0.8-0.9%) [54, 55], with an unusual excess of kappa-restricted cases (96%). Although we have an aged population (mean age 77 years), the difference is substantial. To address this discrepancy, and perhaps a possible over-diagnosis, we evaluated the effect of using a different cut-off for the involved light-chains. We found that the distribution of log-transformed kappa and lambda resembled the normal distribution and we therefore moved the cut-off to the 97.5^{th} percentile. As the 97.5^{th} percentile was at ≈40 mg/L for both kappa and lambda (was before 19.4 mg/L and 26.3 mg/L, respectively) we modified the definition accordingly, although using the same pathological FLC ratio of <0.26 and >1.65 as previously published [133]. We then performed an additional sensitivity analysis on the associations between obesity, diet, and dietary pattern and LC-MGUS in papers I, II, and III, using the same statistical methods and by including the same covariates as described above.

4 Results and discussion

A total of 5,764 participated in the AGES-Reykjavik Study during the years of 2002-2006, thereof 5,725 were included in our analysis (Figure 5), 3,306 (58%) individuals were women and 2,419 were men. The mean age was 77 years (range 66-98). MGUS was identified in 300 (5.2%) subjects, of which 159 (53.0%) were of subtype IgG, 81 (27.0%) were IgM, 27 (9.0%) were IgA, 1 (0.3%) was IgD, and 32 (10.7%) were biclonal. LC-MGUS was identified in 275 (4.8%) subjects, of which 96% were kappa cases. Using the modified definition of LC-MGUS resulted in 52 cases (0.9%), of which 79% were kappa cases. As previous studies have reported [11], we found the prevalence of MGUS to increase with age, it was 5.4% in those older than 70 years old and 7.9% in those older than 85 years old (Table 2). We also found that men were more likely affected than women (Table 2).

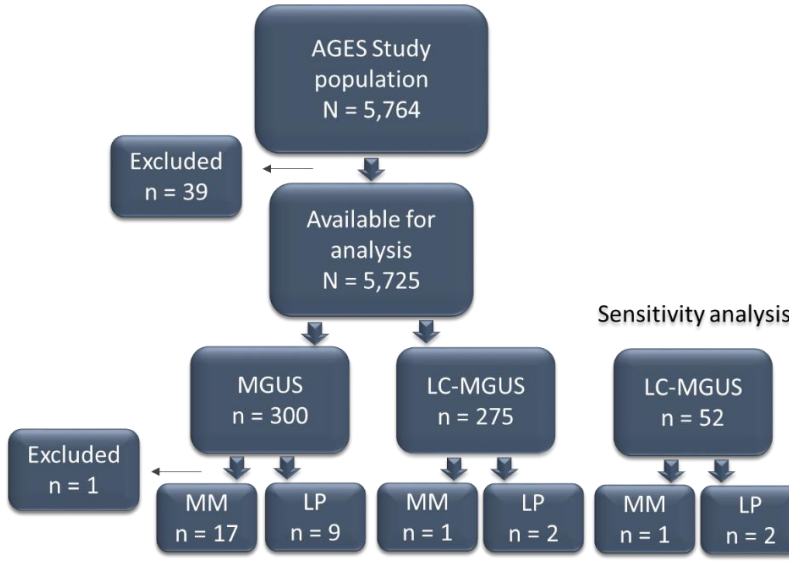

Figure 5 Flowchart of study participants and number of participants affected by MGUS and progression

By cross linking the AGES-Reykjavik Study cohort to the Icelandic Cancer Registry we found that 18 participants progressed to multiple myeloma, of which one from LC-MGUS during a median follow-up of 8 years. Additionally, 11 progressed to other lymphoproliferative diseases (Hodgkin's, and non-Hodgkin's

lymphoma, Waldenström's macroglobulinemia, lymphoid leukemia, chronic lymphocytic leukemia, and acute lymphoblastic leukemia), of which two from LC-MGUS. Furthermore, the individuals diagnosed as having LC-MGUS and progressed to multiple myeloma and other related disease were captured with the modified LC-MGUS definition (Figure 5).

Table 2 Prevalence of MGUS by age and gender

	Men	Women	Total
	number/total number (%)		
70 years and older	150/2,222 (6.8)	129/2,952 (4.4)	279/5,174 (5.4)
85 years and older	26/256 (10.2)	23/361 (6.4)	49/617 (7.9)
Total	159/2,419 (6.6)	141/3,306 (4.3)	300/5,725 (5.2)

4.1 Obesity and monoclonal gammopathies

4.1.1 Obesity and monoclonal gammopathy of undetermined significance

In study I we asked the question whether obesity was a risk factor for MGUS or progression from MGUS to multiple myeloma. We found no association between the 11 tested obesity markers and MGUS or LC-MGUS (see Table 2 in paper I), both in our primary analysis and sensitivity analysis, where we used a modified definition of LC-MGUS. Additionally, by cross-classifying midlife BMI and late life BMI we aimed to test whether history of obesity was a risk factor for MGUS and LC-MGUS but found no associations (see Table 3 in paper I). Previous studies have suggested that the positive association found between BMI and multiple myeloma is explained by an increased risk of MGUS, which in turn puts individuals at risk for developing multiple myeloma [69] but our analyses do not confirm this. To date, three studies have examined the role of obesity in the etiology of MGUS, with conflicting results; one reported no association between BMI and MGUS, although a positive trend was suggested [65], the second reported a positive association in women [69], whereas the third found no association after adjusting for number of lab tests prior to MGUS diagnoses [68]. Since MGUS is usually not screened for, like we do in our study, but identified incidentally through work-up for another unrelated illness, a detection bias may have accounted for the previously reported risk of obesity on MGUS. LC-MGUS is a relatively newly discovered disease and its development and progression is not yet known, however, one study has shown that the risk is increased in first degree relatives of multiple myeloma probands [136].

The dispersion of midlife BMI was very clustered around the mean (25.2 kg/m^2) (Figure 6). Resulting in limited power to categorize BMI according to

commonly accepted classification; underweight (<18.5 kg/m^2), normal weight (18.5-24.9 kg/m^2), overweight (25.0-29.9 kg/m^2), and obesity (≥30.0 kg/m^2) [137]. Approximately 90% of the participants where categorized as either normal weight or overweight, and roughly 9% as overweight and 1% as underweight. Thus, for our analysis in study I, we used the overweight category as cut-off for the upper level of BMI when analyzing the association with MGUS and LC-MGUS.

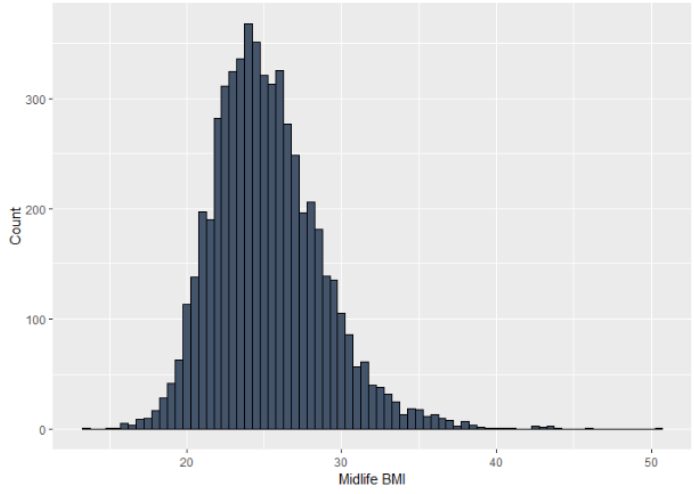

Figure 6 Distribution of midlife BMI

For this thesis we additionally performed an analysis using the commonly accepted classification of midlife BMI, although the underweight category was combined with the normal weight category, and tested its association with MGUS. The results indicated a positive association between the obese category (BMI ≥30kg/m2) and MGUS (Table 3). Similar results were found for LC-MGUS, although not statistically significant.

Table 3 Association between MGUS and midlife BMI using the standard classification

	MGUS			LC-MGUS		
	n (%)	OR (95%CI)	p trend	n (%)	OR (95% CI)	p trend
Midlife BMI						
<25	139 (46.6)	Ref.		128 (46.7)	Ref.	
25-29.9	123 (41.3)	1.06 (0.82-1.37)		115 (42.0)	1.03 (0.79-1.34)	
≥30	36 (12.1)	1.53 (1.05-2.25)	0.03	31 (11.3)	1.42 (0.94-2.13)	0.10

4.1.2 Obesity and progression to multiple myeloma and other lymphoproliferative diseases

The overweight category was also used as cut-off for both midlife and baseline BMI when testing the association with risk of progression to multiple myeloma and related diseases. For that analysis subjects with MGUS and LC-MGUS were combined due to lack of statistical power caused by low number of cases of multiple myeloma and related diseases. We found that high midlife BMI was associated with increased risk of progression from MGUS/LC-MGUS to multiple myeloma and other lymphoproliferative diseases (Table 4), other measures on obesity were not found to be associated with progression (see Table 4 in paper I).

Table 4 Body mass index and risk of multiple myeloma and other lymphoproliferative diseases (LP)

	Multiple myeloma and other LP		
	n	HR	95% CI
Baseline BMI			
<25.0	8	Ref.	Ref.
≥25.0	21	1.30	0.57-2.94
Midlife BMI			
<25.0	8	Ref.	Ref.
≥25.0	21	2.66	1.17-6.05

The association was not explained by known risk factors for progression such as MGUS subtype, M-protein concentration, or FLC ratio as these factors were equally distributed across midlife BMI categories. BMI has consistently been found to be associated with increased risk of multiple myeloma, irrespective of sex, geographical region, assessment of weight and height, follow-up time, or age [40, 138]. Our results are in line with recent studies by Chang et al. (2017) and Veld et al. (2016) that conclude that both overweight and obese individuals with MGUS are at increased risk of progressing [83] and that patients with multiple myeloma have higher abdominal fat and higher fat metabolic activity compared with patients with MGUS [82]. We thus speculate whether the observed risk of multiple myeloma in individuals with obesity is due to increased risk of progression from MGUS, rather than increased risk of MGUS. Our results are limited by low number of cases, however, the consistently high estimate both when multiple myeloma was analyzed with and without other lymphoproliferative diseases, strengthens the notion that obesity plays a role in MGUS progression. Our results also indicate that obesity might be the first modifiable risk factor for progression from MGUS to multiple myeloma.

In our cohort, none of the exposures measured at study baseline, at time of MGUS screening, were associated with progression from MGUS to multiple myeloma and other lymphoproliferative diseases. We do have an elderly population, with a mean age of 77 years and a range of 66-98 years, and we do not know for how long MGUS had been present in our subjects before entering the study. This is true for most studies on MGUS since it is difficult to determine the true incidence due to the asymptomatic nature of the disease. According to a US-based screening study by Therneau at al., when first recognized, MGUS has possibly been present in an undetected state for a median duration of over 10 years. The authors also estimate that 56% of women and 55% of men over the age of 70 years have had MGUS for over 10 years when first recognized, including 28% and 31%, respectively, for over 20 years [139]. The mean age of individuals with MGUS in our cohort is 79 years, ranging from 66-97 years, and we can expect, based on the results by Therneau et al., that a large proportion of our subjects have had the condition for over 20 years. For that reason, the onset of MGUS was possibly closer in time to the midlife BMI assessment than the late life assessments, indicating that in a screened population with high mean age, assessments closer to the true onset of MGUS might be a better indicator for progression than assessment at screening.

4.2 Dietary habits and monoclonal gammopathies

4.2.1 Main findings of study II and study III

In study II and III we tested whether diet was associated with MGUS, LC-MGUS, or progression to multiple myeloma and other lymphoproliferative diseases. In study II we focused on single food analyses from three different periods; adolescence (14-19 years), midlife (40-50 years), and late life (at AGES study entry) and in study III we used factor analysis to identify dietary patterns from the adolescent period to use as exposure variable. The main findings of study II and III were that adolescent and late life fruit intake were inversely associated with MGUS and progression to multiple myeloma and other lymphoproliferative diseases (OR = 0.62, 95% CI 0.41-0.95 and HR = 0.45, 95% CI 0.21-0.96, respectively). Midlife intake of whole wheat bread was inversely associated with MGUS (OR = 0.75, 95% 0.57-0.99), and the Traditional early 20th century diet was inversely associated with both MGUS and LC-MGUS (Table 6). Lastly, fish was unexpectedly not associated with MGUS, LC-MGUS, or progression.

4.2.2 Changes in dietary habits

The participants in the AGES-Reykjavik Study were born during the years of 1907 to 1935 and at study entry, during the years of 2002 to 2006, they gave retrospective information on commonly eaten food from the adolescent and

midlife periods. Due to the limited infrastructure in Iceland during the early 20th century, many regions were relatively isolated. There was a considerable variation in diet across the country and the diet was characterized by locally produced foods available on site [129, 130], although there was a considerable importation from Denmark, especially on rye, barley, and oats [124]. In the rural areas consumption of salted or smoked meat and fish, milk and milk products, and rye bread was very high, but very little consumption of fruit was noticed. Fish and fish oil were most commonly consumed in the sea villages and in the capital area. We also see in our data how the dietary habits shifted throughout the period (Table 5).

Table 5 Dietary habits of participants from three different time periods

	Adolescence n (%)	Midlife n (%)	Late life n (%)
Fish			
≤ 2 portions p/w	2,574 (48.8)	633 (12.0)	1,431 (27.1)
> 2 portions p/w	2,696 (51.2)	4,646 (88.0)	3,851 (72.9)
Fish oil			
less than weekly	2,527 (47.7)	2,046 (38.7)	1,566 (29.6)
weekly or more	2,773 (52.3)	3,247 (61.2)	3,725 (70.4)
Salted/smoked fish*			
3 times a month or less	2,452 (46.5)	3,589 (67.8)	5,047 (95.2)
once p/w or more	2,824 (53.5)	1,704 (32.2)	254 (4.8)
Meat			
2 times p/w or less	1,883 (35.6)	2,008 (37.9)	1,966 (37.0)
3 times p/w or more	3,408 (64.4)	3,289 (62.1)	3,344 (63.0)
Salted/smoked meat*			
3 times a month or less	3,452 (65.3)	3,899 (73.7)	5,010 (94.4)
Once a week or more	1,832 (34.7)	1,393 (26.3)	295 (5.6)
Milk and milk products			
less than daily	1,202 (22.7)	2.146 (40.6)	2,672 (50.4)
daily	4,102(77.3)	3,143 (59.4)	2.629 (49.6)
Fruit			
2 times p/w or less	4,535 (85.8)	3,637 (68.7)	1,466 (27.6)
3 times p/w or more	753 (14.2)	1,657 (31.3)	3,840 (72.4)
Vegetables			
2 times p/w or less	3,911 (73.9)	3,425 (64.8)	2,760 (52.0)
3 times p/w or more	1,381 (26.1)	1,858 (35.2)	2,543 (48.0)
Rye bread/flatbread			
less than daily	2,742 (51.9)	3,615 (68.2)	2,374 (44.9)
daily	2,539 (48.1)	1,686 (31.8)	2,918 (55.1)
Sausage/liver			
less than weekly	1,394 (26.3)	2,557 (48.3)	1,336 (25.2)
weekly or more	3,908 (73.7)	2,742 (51.7)	3.961 (74.8)
Oatmeal/musli			
2 times p/w or less	2,377 (44.3)	3,197 (60.5)	2,767 (52.1)
3 times p/w or more	2,940 (55.7)	2,091 (39.5)	2,542 (47.9)
Potatoes			
less than daily	543 (10.3)	830 (15.7)	2,134 (40.2)
daily	4,750 (89.7)	4,469 (84.3)	3,173 (59.8)
Whole wheat bread			
4 times p/w or less		1,677 (31.7)	1,346 (25.4)
5 times p/w or more		3,609 (68.3)	3,953 (74.6)

*Due to limited power, different cut-off for salted/smoked fish and salted/smoked meat (once a month or more) was used when analyzing late life intake and risk of progression to multiple myeloma

According to Icelandic national surveys the fish intake of Icelanders decreases drastically during the 20th century, although it was still very high by international comparison [124, 125, 129]. That is however, not reflected in our data as we see in increase throughout our period. Two reasons might explain the discrepancy; firstly, the aging of the population under study, those who had high fish intake as teenagers still had high intake throughout life, and secondly, during the World War II British forces occupied the country and with them came employment and income and people started moving from the countryside to the towns, especially Reykjavik and thereby possibly increasing the intake of our participants [124]. As a result of both increase in domestically produced vegetables and increased importation of both vegetables and fruit during the 20th century [124] we notice a dramatic increase in intake of fruit and vegetables throughout the period (Table 5). We additionally notice a decrease in consumption of the food commonly eaten in the rural areas such as salted or smoked fish and meat (Table 5), possibly due to the previously mentioned changes in the nation's residency.

4.2.3 Diet and monoclonal gammopathy of undetermined significance

In study II we tested the association between MGUS and 12 different foods and food groups from the adolescent period and 13 from the midlife period. Whole wheat bread intake was not assessed in the adolescent FFQ due to limited availability during the early 20th century in Iceland [124]. We found an inverse association between midlife whole wheat bread intake and adolescent fruit intake and MGUS (OR = 0.75, 95% CI 0.57-0.99 and OR = 0.62, 95% CI 0.41-0.95, respectively), other foods from the adolescent and midlife periods were not found to be associated with MGUS (see Table 2 and Table 3 in paper II). However, we did not find the Healthy diet, that includes fruit, to be associated with MGUS in study III, as we expected based on the results from study II (Table 6). However, we found the Traditional early 20th century diet to be inversely associated with MGUS (Table 6). Some of the food components in the Traditional pattern have previously been found to increase the risk of various types of cancer, such as red and processed meat [16]. Therefore, these results were somewhat surprising. None of individual food components in the Traditional pattern were found to be associated with MGUS in study II. Thus, it seems, that the combined effect of the dietary pattern is more powerful than its individual components. The biological mechanism for these findings is unknown, the pattern might primarily be an indication of old traditional habits in Iceland in general, suggesting an effect of environmental or behavioral factor on MGUS pathogenesis. When interpreting these findings, we found that 56% of those growing up in the rural area of Iceland, mostly farmers, were in the highest tertile of adherence to that pattern,

but only 20% and 29% of those living in the Reykjavik capital area and the sea villages, respectively. Adjusting for residency did not affect our results, we therefore speculate whether residency is a proxy for some other risk factors, such as physical activity. However, adjusting for physical activity did not change the results. Nevertheless, farming required a lot of physical strength, which is not captured within the physical activity question included in the AGES-Reykjavik Study, but this population could reflect a physically healthy population that could affect disease outcome.

Table 6 Association between dietary patterns from the adolescent period and its indvidual food components and MGUS and LC-MGUS

Dietary pattern	All MGUS OR (95% CI)	MGUS OR (95% CI)	LC-MGUS OR (95% CI)
Traditional early 20th century diet*	0.67 (0.53-0.85)	0.68 (0.50-0.93)	0.67 (0.49-0.92)
Salted/smoked meat	0.99 (0.97-1.01)	0.82 (0.61-1.11)	0.95 (0.97-1.29)
Salted/smoked fish	0.99 (0.97-1.01)	0.95 (0.71-1.26)	0.89 (0.66-1.21)
Blood sausage/liver sausage	0.99 (0.97-1.01)	0.86 (0.64-1.18)	0.86 (0.62-1.19)
Rye bread	1.00 (0.98-1.01)	0.94 (0.71-1.23)	1.00 (0.75-1.34)
Milk & milk products	0.99 (0.97-1.01)	0.91 (0.67-1.24)	0.85 (0.61-1.19)
Oatmeal	1.00 (0.98-1.02)	1.09 (0.83-1.44)	0.93 (0.70-1.24)
Potatoes	0.99 (0.96-1.02)	0.81 (0.55-1.20)	1.04 (0.67-1.63)
Healthy diet*	0.92 (0.73-1.16)	1.20 (0.88-1.59)	0.67 (0.48-0.94)
Fruit	0.98 (0.95-1.00)	0.62 (0.41-0.95)	0.84 (0.54-1.32)
Vegetables	1.00 (0.98-1.02)	1.17 (0.87-1.57)	0.80 (0.57-1.13)
Fish on bread or in salad	0.98 (0.96-1.01)	0.96 (0.60-1.51)	0.68 (0.39-1.20)

*Highest tertile of intake compared to the lowest tertile. Models are adjusted for age and sex, and the individual food components are additionally adjusted for all the other food items.

4.2.4 Diet and light chain monoclonal gammopathy of undetermined significance

As described above the prevalence of LC-MGUS was much higher in our cohort than has been previously reported [54, 55]. Although the population has high mean age, the difference is substantial. We therefore performed a sensitivity analysis on the association with diet using a modified definition of LC-MGUS. In the primary analysis in study II we found that adolescent fish intake was positively associated with LC-MGUS (OR = 1.39, 95% CI 1.06-1.83), which was not confirmed in the sensitivity analysis. Other foods from the adolescent period were not found to be associated with LC-MGUS (see Table 2 in paper II). In study III we found that the both the Traditional and the Healthy diet was inversely associated with LC-MGUS (Table 6). The association with the Traditional diet was confirmed in our sensitivity analysis, but not the association with the Healthy diet. Other inconsistencies between the primary and sensitivity analysis were also noticed. The reason for the attenuated associations could be due to loss of

power in our analysis as the number of LC-MGUS cases dropped from 275 to 52, and it is therefore hard to draw conclusions from these results. None of the foods from midlife was found to be associated with LC-MGUS (see Table 3 in paper II).

4.2.5 Late life diet and progression

When analyzing the 13 food items from the late life period we found, based on small number of cases, that fruit intake reduced the risk of progressing from MGUS to multiple myeloma and other lymphoproliferative diseases (HR = 0.45, 95% CI 0.21-0.96). That is consistent with the findings from the adolescent period, although we did not see the same from the midlife period. The literature regarding the role of fruit in the etiology of multiple myeloma is limited, reporting no association [17, 20-22]. However, there are major methodological differences between these studies and ours that could explain the discrepancy. The potential biological mechanism behind the association could be the anti-carcinogenic effect of vitamins C [140], although the cancer-preventive effect of vitamin C has been a subject of controversy ever since suggested in the 1930s [141]. Other foods from the late life period were not found to be associated with risk of progressing from MGUS/LC-MGUS to multiple myeloma and other lymphoproliferative diseases (see Table 4 in paper III).

4.3 Methodological issues

The goal of most epidemiological studies is to attain precise and valid estimate of the frequency of a disease or of the association between a possible cause and an occurrence of a disease. Errors in the estimate are traditionally classified as either random or systematic. Random error is often linked with chance or random variation and is derived from errors in sampling and measurements and biological variation. Systematic errors in epidemiology are often referred to as biases and are traditionally split in three categories; selection bias, information bias, and confounding. The opposite of random error is precision and the opposite of systematic error is validity [142]. The validity of a study is often separated into external validity and internal validity. External validity represents the generalizability of a study and how the results pertain to people outside the study population. Internal validity refers to how well an experiment is done. It represents the causal relationship between the subject variables and how the results pertain to the members of the source population [142]. When designing an epidemiological study, it is important to try to reduce both systematic and random of errors so that the estimate can be as precise and valid as possible. In our studies we used population-based data to test the association between possible risk factors for MGUS and progression to multiple myeloma and related diseases. As for most cohort studies, our results are subjected to bias that could potentially affect precision and both internal and external validity.

4.3.1 Selection bias

Selection bias results from the procedure that is used to select study participants and from factors that influence participation. As a result, the relation between the exposure and outcome of interest is different for those who participate in the study than for those who were eligible for the study [142]. Our study is possibly subjected to selection bias. Even though the cohort is population-based it is possible that those who agreed to participate differ from those who did not participate. Firstly, those who participated in the original Reykjavik Study might vary in the sense that they possibly represent a population that is healthier than the population under observation. Even though the participants did not self-volunteer but were chosen randomly from the population under study, those who consented to participated might be more health conscious, and therefore have more favorable distribution of risk factors. Secondly, those who were eligible for the AGES-Reykjavik Study were the previous participants of the Reykjavik Study. When the AGES-Reykjavik Study was initiated a large fraction of the participants were deceased and the cohort might therefore include healthy individuals, resulting in a survival bias. The effect estimate for our exposures could therefore be attenuated. Although selection bias mainly addresses internal validity of a study, survival bias can influence the generalizability (i.e. external validity) of the study.

4.3.2 Information bias

Information bias occurs after the subjects to be compared have been identified. The bias is caused by an error in the measurements of the needed information and is a threat to internal validity [142]. MGUS is asymptomatic and is most often diagnosed by chance during clinical work-up of other unrelated illnesses, therefore detection bias is common when studying both risk factors and progression or survival of MGUS subjects. Detection bias can cause MGUS patients to have unfavorable distribution of risk factors or co-morbidities, factors that lead up to the MGUS diagnosis, compared non-MGUS subjects. However, this bias can be avoided by screening for MGUS, as we do in our study. Misclassification of exposure (or outcome) affects most epidemiological studies. Misclassification can be either differential or non-differential. Differential misclassification can happen when status of exposure is collected after the disease of interest has occurred, the exposure is then classified differently for those with and without the disease. This type of misclassification can either exaggerate or underestimate an association and is often referred to as recall bias. With non-differential misclassification the exposure is misclassified, but the misclassification does not depend on disease status and the bias is therefore in the direction of the null value [142, 143]. However, one can only assume non-

differential misclassification on binary exposure variables, and when collapsing continuous or categorical variables into fewer categories the misclassification can be changed from non-differential to differential [142]. Dietary data in the AGES-Reykjavik Study cohort was collected using an interview administered FFQ. Participants had to recall the most commonly eaten foods in Iceland during their adolescence and midlife periods and give information on current intake. Our data is therefore subjected to misclassification. However, since our participants did not know their disease statues (presence or absence of MGUS) during dietary assessment the misclassification is most likely non-differential. Nevertheless, the bias could have been pushed away from the null value when collapsing intake categories during statistical analysis. It can be challenging recalling dietary intake many decades back in time. Nevertheless, a study on food related memory concluded that memory of foods does not necessarily decline over time [144]. Recalling many decades back in time can be just as accurate as more recent memory, especially with regards to foods eaten daily or very rarely. When it comes to studies with the aim to test for example whether groups with higher intake are more likely to get a specific disease than those with lower intake, then it is the ranking within the cohort that is important. Equal over- or underreporting (non-differential misclassification) should not affect results. [144]. The diet of Icelanders during the first half of the 20th century was simple with little variability [124], which should make recalling easier and less sensitive to bias. Another type of information bias that might be present in our data is a result of a biased follow-up time in the progression analysis. We do not know the true time of MGUS onset. The follow-up starts at screening (AGES-Reykjavik Study entry) and ends at diagnosis of multiple myeloma or of other lymphoproliferative diseases, but MGUS has most likely been present in most of our MGUS patients for a period of time [139], particularly in the older subjects, that might result in shorter follow-up time. But since prevalence of both MGUS and multiple myeloma and related diseases increases with age then adjusting for age should reduce the effect of this bias.

4.3.3 Confounding

Rothman defines confounding as “confusion, or mixing, of effects: the effects of the exposure is mixed together with the effect of another variable, leading to bias” [143]. To be considered a confounder the variable must be associated both with the exposure and outcome under study, but it must not be affected by the exposure or the disease, in particular it cannot be in the causal pathway between the exposure and the outcome [142, 143]. Confounding can only be addressed if information on the confounding variable has been collected. The AGES-Reykjavik Study data collection was extensive and included collection of multiple health related variables and one of its main objective was to explore the role of

behavioral and environmental factors on diseases that manifest late in life [112]. As stated in chapter 3.6 we considered potential confounder in our analysis. MGUS and multiple myeloma and related diseases are highly dependent on age and sex and therefore these factors were included in all our models. In study I, on the association between obesity and MGUS and MGUS progression, we additionally tested the confounding effect of height in models where height is not considered in the exposure variable, such as weight, waist circumference, and CT measures. Height was not included in the final model as it had no effect on the estimates. In study II we additionally tested the confounding effect of BMI and physical activity in the association between different foods or food groups and MGUS and MGUS progression. Both factors are not obvious confounders. They are both associated with the exposure, but not necessarily the outcome. We had, for example, previously found that BMI was not associated with MGUS, and there is no solid evidence with regards to physical activity. Both factors were not found to confound the association and were therefore not included in final models. In study III we got results that were surprising with no obvious biological mechanism. We tried to find a possible explanation for the observed association, by additionally adjusting for BMI, physical activity, education, and residency, without any obvious result. We therefore reported results from both models, age-adjusted and fully adjusted. Family history of lymphoproliferative diseases is a possible confounder that we were unable to account for in our analysis due to lack of information. Family history is associated with increased risk of MGUS and multiple myeloma [13, 67] and it is possibly also associated with the exposures as relatives of patients with such diseases may be more health conscious and more likely to take positive and favorable health related decisions. That could have influenced our estimates.

4.3.4 Multiple testing

Multiple testing arises when statistical analysis involves multiple statistical tests among numerous exposures and outcomes. To analyze each association the CI is usually set at 95%, which means that if we test 100 associations, we should expect five to miss target on the true value [142]. This type of error is called a type I error (coming about a significant result by pure chance, false positive). A common method to account for multiple comparison is the Bonferroni method. The Bonferroni correction is a conservative test that, although protects from type I error, is vulnerable to type II errors (failing to reject the null hypothesis when you should in fact reject the null hypothesis, false negative) [142]. Study II is particularly vulnerable to type I error and we cannot rule out the option that our results are due to chance. Since little is known on the association between our exposure (diet) and outcome (MGUS and multiple myeloma), study II can be considered a hypothesis-generating study. That is when a researcher explores a

set of data searching for associations (risk factors) and then afterwards proposes a hypothesis to be tested in subsequent studies [145]. The nature of our study was to seek risk factors for MGUS and MGUS progression and by adjusting for multiple comparison we would risk missing out on possible risk factors (type II error). So, it was important for the interpretation of the findings to not adjust for multiple comparison, and risk type II error. Additionally, as Rothman states, study data are not random facts but real observations and should not be punished by missing out on essential findings [146].

4.3.5 Validation of the diet

As discussed in chapter 3.2.2 on validation, the FFQ has been validated for the midlife and late life periods, showing acceptable validation for most food items, but not all [127, 128]. Adolescent diet has not and cannot be validated. However, it shows similar distribution of intake between types of residency, i.e. rural, seaside and capital area, as did a household study from 1939 [129, 130], the time when most of our participants were adolescents. The reasons for lack of validity of some of the food items in late life are possibly the inability of the reference method (3-day food record) to adequately reflect intake of foods that are consumed infrequently. Also, there may be lack of intake distribution for some items. Due to those reasons, and due to the hypothesis-generating nature of the study, we did not eliminate any food items from our analysis that lacked validity based on these reference methods.

The cut-offs for upper intake of food consumption were chosen based on intake distribution. Considering that the same cut-off could be used for all three time periods for consistency and comparison of each food item. However, due to dramatic changes in dietary habits of Icelanders during the 20th century this was not possible for all food items. Intake of salted or smoked fish and meat dropped drastically and therefore the same cut-off could not be used. For the adolescent and midlife periods, the cut-off was at once a week, but for the late life period it was at once a month.

4.3.6 Strengths of the study

The major strength of our studies is the population-based cohort design of the AGES-Reykjavik Study cohort, with extensive information on covariates. The use of the nationwide Cancer Registry to identify cases of multiple myeloma and other lymphoproliferative diseases is additionally a major strength. It ensures detailed and valid assessment of our outcome with virtually complete follow-up as it has high diagnostic accuracy and completeness (99%) [134] and all participants had equal access to the public health care system at study entry. Screening for MGUS adds to the strength of our study. The interpretation of the

SPEP was performed by two lab experts blinded to all demographics of the study participants. As stated above, MGUS is usually discovered by chance during clinical work-up of other causes that can bias the association with possible risk factors. We used various and precise measures to assess obesity. BMI is a limited measure as it does not take into consideration the difference between lean and fat mass, although it highly correlated with the other measures (r = 0.37-0.95) and has been shown to have similar ability to predict diseases [116]. Nonetheless, by using multiple methods deals with possible limitations of single method use. The consistency in the findings throughout the assessment methods also strengthens our findings. The utilization of validated FFQ were majority of the questions (although with some exceptions) was found suitable to rank individuals according to intake is a strength. The ability to study dietary intake throughout the lifespan is a major strength. Studying early life exposure poses some challenges but is very important since many diseases manifest early in life. The literature is limited with regards to lifetime dietary intake and association with late life health outcomes and we believe our study is unique in that aspect. Lastly, the use of empirically derived method (PCA) to identify dietary patterns in study III, as a complementary approach to single food analysis, gives better understanding of the complex association between diet and MGUS and MGUS progression.

5 Summary and conclusions

Three studies form the foundation of this theses, all using data from the population-based Age, Gene/Environment Susceptibility-Reykjavik Study (N=5,764). The aim of study I was firstly, to explore the association between various obesity markers and MGUS and secondly, to analyze its effect on risk of progressing from MGUS to multiple myeloma and related diseases. We found that obesity was not associated with MGUS but was a risk factor for progression. Indicating that the previously observed associations between obesity and multiple myeloma is due to its effect on progression from MGUS rather than affecting MGUS onset. The aim of study II was to identify diet related risk factors for MGUS and progression from MGUS to multiple myeloma and related diseases, using a validated FFQ from three different time periods (adolescence, midlife, and late life). Limited literature exists on the association to guide hypothesis; therefore, all foods were tested within a hypothesis-generating study. We found that adolescent fruit intake and midlife whole wheat bread intake were inversely associated with MGUS and that late life fruit intake reduced risk of progressing from MGUS to multiple myeloma and related diseases. The aim of study III was to analyze if empirically derived dietary patterns from the adolescent period were associated with MGUS. To our surprise, we found that a Traditional diet from the early 20th century, characterized by high intake of salted or smoked meat and fish, blood or liver sausage, rye bread, milk and milk products, oatmeal, and potatoes was inversely associated with MGUS. The second and third studies indicate a possible role of diet or diet related proxy in myelomagenesis.

This thesis highlights the potential role of behavioral and environmental factors in myelomagenesis and indicate that the risk of multiple myeloma can be modified by taking favorable lifestyle related decisions. Our population-based data suggest that the previously observed association between obesity and multiple myeloma is due to its effect on MGUS progression, and our study is unique in that aspect as it disentangles the associations between MGUS and multiple myeloma with regards to possible risk factors within the same study. Diet has up until now not been strongly linked with multiple myeloma, but our studies indicate a possible effect of diet on MGUS and multiple myeloma and raises questions that need to be further studied within large-scale population-based cohort design.

6 Future studies

Our data provide important evidence for the role of obesity and diet in myelomagenesis, but we remain cautious in our interpretation. Causal inference cannot be made from these studies alone and more studies are needed, especially with regards to diet. Firstly, to explore possible biological and genetic mechanism for our results, and secondly, to test possible hypothesis that can be generated from our findings, such as the effect of fruit on MGUS and MGUS progression. Our study population consists of a relatively isolated population, with very distinct dietary habits, therefore, the challenges of studying complex multicultural diet were limited. However, that could affect generalizability of our findings. Additionally, due to the low prevalence of multiple myeloma a larger population size is needed to confirm our findings. The findings from all three studies need to be substantiated by obtaining more detailed information on dietary intake and other lifestyle related factors, preferably within a screened prospective study design. As mentioned previously, when screening for MGUS a less biased inference can be made with regards to possible risk factors for MGUS and progression to multiple myeloma. Recently a prospective population-based screening study for MGUS was initiated in Iceland (iStopMM). All residents of Iceland born in 1975 or earlier were invited to participate in the study (N ≈ 120,000) and health data will be collected at regular intervals. Studying possible lifestyle-related risk factors, for MGUS progression specifically, within such a large-scale study could give valuable information with regards to modifiable risk factors for multiple myeloma.

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Paper I

REGULAR ARTICLE

blood advances

Obesity and risk of monoclonal gammopathy of undetermined significance and progression to multiple myeloma: a population-based study

Marianna Thordardottir,[1] Ebba K. Lindqvist,[2] Sigrun H. Lund,[1] Rene Costello,[3] Debra Burton,[3] Neha Korde,[4] Sham Mailankody,[4] Gudny Eiriksdottir,[5] Lenore J. Launer,[6] Vilmundur Gudnason,[1,5] Tamara B. Harris,[6] Ola Landgren,[4] and Sigurdur Y. Kristinsson[1,2]

[1]Faculty of Medicine, University of Iceland, Reykjavik, Iceland; [2]Division of Hematology, Department of Medicine, Karolinska University Hospital and Karolinska Institutet, Stockholm, Sweden; [3]Multiple Myeloma Section, National Cancer Institute, National Institutes of Health, Bethesda, MD; [4]Myeloma Service, Division of Hematologic Oncology, Memorial Sloan Kettering Cancer Center, New York, NY; [5]Icelandic Heart Association, Kopavogur, Iceland; and [6]National Institute on Aging, National Institutes of Health, Bethesda, MD

Key Points

- Obesity is not associated with MGUS or LC-MGUS.
- High body mass index during midlife is associated with increased risk of progressing from MGUS and LC-MGUS to MM and other LP diseases.

All multiple myeloma (MM) cases are preceded by the premalignant state monoclonal gammopathy of undetermined significance (MGUS). Results from previous studies show a positive association between obesity and MM; however, the association between obesity and MGUS is controversial. The aims were to determine (1) if obesity is associated with an increased risk of MGUS and light-chain MGUS (LC-MGUS) and (2) whether obesity is associated with a higher risk of progression to MM and other lymphoproliferative (LP) diseases. Data from the population-based Age, Gene/Environment Susceptibility–Reykjavik Study (N = 5764) were used. We performed serum protein electrophoresis and serum free light-chain assay on all subjects to identify MGUS and LC-MGUS cases. We included 11 different measures on current and previous obesity in our analysis. Logistic regression and Cox proportional-hazard regression were used to analyze the associations. A total of 300 (5.2%) MGUS and 275 (4.8%) LC-MGUS cases were identified. During a median follow-up of 8 years, 18 had progressed to MM and 11 to other LP diseases. We found no association between the 11 obesity markers and MGUS or LC-MGUS (odds ratios 0.81 to 1.15 for all 11 variables in both conditions). Interestingly, we found that high midlife body mass index increased risk of progression to MM and other LP diseases (hazard ratio, 2.66; 95% confidence interval, 1.17-6.05). To conclude, obesity was not associated with MGUS. However, we found overweight/obesity to be a risk factor for progression from MGUS to MM and other LP diseases, suggesting that obesity plays a role in the transformation of MGUS to MM.

Introduction

Overweight and obesity have consistently been recognized as risk factors for many common cancers.[1-4] The World Health Organization (WHO) recently concluded that there is now sufficient evidence for the effect of weight on 13 types of cancers, including multiple myeloma (MM).[5,6] MM is a chronic B-cell disorder characterized by a monoclonal proliferation of plasma cells in the bone marrow.[7] All cases of MM are preceded by the premalignant state monoclonal gammopathy of undetermined significance (MGUS),[8,9] which is an asymptomatic condition characterized by the presence of an M-protein in serum without evidence of MM or other lymphoproliferative (LP) diseases.[10] The prevalence of MGUS increases with age and is reported to be found in ~5% of individuals older than 70 years.[11] The average risk of progression from

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MGUS to MM is estimated to be 1% per year.[12,13] Light-chain MGUS (LC-MGUS) has recently been described as a precursor condition to light-chain MM.[14,15] LC-MGUS is defined as an abnormal free light-chain (FLC) ratio with no expression of heavy chains, along with an increased concentration of the light chain involved, and its prevalence has been estimated to be 0.7% to 0.8%.[14,15]

The etiology of MGUS and LC-MGUS is to a large extent unknown. However, researchers have found higher risk of MGUS among males,[11] blacks,[16,17] those with family history of MGUS and related diseases,[18] and those with prior personal and family history of immune-related conditions.[19,20] Obesity has been identified as a risk factor for MM, with[6,21,22] 2 meta-analyses finding a 10% to 20% increased risk of MM for a 5 kg/m^2 increase in body mass index (BMI).[21,22] However, BMI is a limited measurement as it does not provide information on fat distribution, which is of importance as many of the complications associated with obesity have been shown to be closely related to abdominal obesity.[23,24] A recent pooled analysis of 20 prospective studies found that waist circumference is a risk factor for MM mortality and that BMI in early adulthood plays an important role later in life regarding MM mortality, particularly in women.[25] In addition, a recent cross-sectional study (n = 72) found that patients recently diagnosed with MM had higher abdominal fat compared with patients with MGUS, indicating that this parameter might serve as a biomarker for progression from MGUS to MM.[26]

The biological mechanisms through which obesity might influence MM etiology are not yet established. Suggested mechanisms are effects of interleukin-6 (IL-6), insulin-like growth factor 1, and adiponectin. IL-6 is directly associated with obesity, ~15% to 35% of total IL-6 is produced by adipose tissue, and IL-6 is a growth factor in MM.[27] Obesity can lead to elevated levels of bioavailable insulin-like growth factor 1, which may increase MM cell proliferation and inhibit apoptosis.[28] Additionally, levels of adiponectin are lower in obese individuals,[29] and a recent study showed that higher levels of adiponectin lowered the risk of MM[30] as well as progression from MGUS to MM.[31]

To date, only 2 studies on the association between obesity and MGUS have been conducted. A study from the United States based on 60 cases found a twofold increased risk of MGUS in obese (BMI >30 kg/m^2) vs nonobese women.[32] Another screening study from the United States, based on 365 cases of MGUS identified through the National Health and Nutritional Examination Survey, did not find an association.[33] Both studies relied only on BMI as a marker for obesity. However, obesity is an outcome of many different factors, both physiological and behavioral, and could therefore be a proxy for these factors that might independently have an effect on MGUS risk. The association between obesity and LC-MGUS has, to our knowledge, not been studied.

The aim of this study was to analyze the association between 11 different obesity assessment methods and MGUS and LC-MGUS using data from a large population-based cohort, and, furthermore, to analyze if obesity is a risk factor for progression from MGUS or LC-MGUS to MM and other LP diseases.

Methods

Study population

This study is based on participants from the Age, Gene/Environment Susceptibility–Reykjavik Study (AGES-RS), which is a continuation of the population-based Reykjavik Study.[34] The Reykjavik Study began recruiting in 1967 a sample of more than 30 000 residents of Reykjavik born in 1907 to 1935. In 2002, the AGES-RS started recruiting 5764 of the surviving members. A detailed description of the study and data collection has been published previously.[34]

All participants in the AGES-RS signed an informed consent form, and the study was approved by the Icelandic National Bioethics Committee (VSN-00-063-V35), the Icelandic Data Protection Authority, and the Institutional Review Board of the National Institute on Aging in the USA.

Material and measures

A 0.5-mL aliquot from the serum collected at AGES-RS study baseline (2002-2006) was obtained for each study subject. Each sample tube was labeled only with the participant's coded ID number and the specimen collection year. All specimens were shipped on dry ice to the Multiple Myeloma Research Laboratory at the National Cancer Institute, where protein assays were performed. To identify MGUS cases in the AGES-RS cohort, we performed conventional agarose-gel serum protein electrophoresis (SPEP) for all subjects (Helena Laboratories, Beaumont, TX). Samples with an equivocal or definite M-protein present on SPEP were subjected to serum protein immunofixation for confirmation and typing of the M-protein.[11] Serum FLC assay (FREELITE; The Binding Site Ltd., Birmingham, UK) was performed on all samples.[35] All testing and interpretations were done by 2 individuals (R.C. and D.B.) blinded to all demographics and other details pertaining to the samples being tested. The results were merged with the data from the original AGES-RS cohort.

MGUS cases were defined as having 1 or several M-protein bands on SPEP and an M-protein concentration <30 g/L.[36] The criteria for LC-MGUS were having no M-protein band visible on SPEP and a pathological FLC ratio (<0.26 or >1.65) on FLC analysis in combination with an increased concentration of the light chain concerned (f-κ >19.4 mg/L, f-λ >26.3 mg/L).[37]

We included 11 different measures on obesity in our analysis. Baseline measures were weight (kg), BMI (kg/m^2), percent body fat (%), fat (kg), total body fat (cm^2), visceral fat (cm^2), subcutaneous fat (cm^2), and 2 versions of abdominal circumference (cm). We also included self-reported lifetime maximum weight and measured midlife BMI (obtained from the Reykjavik Study data). Weight was measured using a digital scale, and height was measured using a stadiometer. Both measures were performed multiple times, and mean numbers found. Percent body fat and fat were calculated from bioelectric impedance (Xitron Hydra ECF/ICF Bio-Impedance Spectrum Analyzer), a commonly used method for estimating body composition. Computed tomography (CT) imaging of the abdomen at the level of the L4/L5 vertebrae was performed to calculate total body fat, visceral fat, subcutaneous fat, and abdominal circumference.[34] Abdominal circumference was also conventionally measured[38] multiple times, and a mean number found. Midlife BMI was calculated from weight and height measured at enrollment into the Reykjavik Study. The mean age of participants at Reykjavik Study entry was 53.3 years for women and 52.1 years for men.[39]

A total of 5764 persons were enrolled in AGES-RS. A total of 39 (0.7%) were excluded from analysis in this study, 21 subjects because of previous LP diseases, 16 because of missing blood

Table 1. Characteristics of the study participants

Characteristics	Without MGUS, n = 5150 (93.9%)	MGUS, n = 300 (5.2%)	LC-MGUS, n = 275 (4.8%)	MM, n = 18 (3.1%)*	MM and other LP diseases, n = 29 (5.1%)*
Sex, n (%)					
Female	3046 (59.1)	141 (47.0)	119 (43.3)	10 (55.6)	14 (48.3)
Male	2104 (40.9)	159 (53.0)	156 (56.7)	8 (44.4)	15 (51.7)
Mean age (range), y	76.8 (66-98)	78.3 (67-93)	79.4 (66-97)	77.8 (69-87)	77.4 (68-87)
BMI, mean (SD), kg/m^2	27.0 (4.5)	26.7 (4.1)	27.0 (4.5)	27.1 (2.4)	26.5 (2.8)
BMI, % (n)					
<25.0	1716 (34.0)	101 (34.1)	93 (34.2)	5 (27.8)	8 (27.6)
25.0-29.9	2222 (43.6)	146 (49.3)	121 (44.4)	10 (55.6)	18 (62.1)
≥30.0	1154 (22.7)	49 (16.6)	58 (21.3)	3 (16.7)	3 (10.3)
BMI midlife, mean (SD), kg/m^2	25.2 (3.6)	25.5 (3.7)	25.6 (3.8)	26.2 (2.7)	26.2 (3.2)
BMI midlife, n (%)					
<25	2686 (52.4)	139 (46.7)	128 (46.7)	5 (27.8)	8 (27.6)
≥25	2442 (47.6)	159 (53.3)	146 (53.3)	13 (72.2)	21 (72.4)
Weight, mean (SD), kg	75.3 (1.7)	75.4 (14.2)	76.4 (15.6)	77.2 (13.5)	76.8 (13.7)
Max weight, mean (SD), kg	80.7 (15.5)	82.4 (15.2)	83.1 (16.1)	85.4 (13.7)	85.7 (13.8)
Percent body fat (BIA), mean (SD), %	29.0 (7.9)	26.8 (8.4)	26.9 (7.7)	28.7 (7.6)	26.8 (7.9)
Fat (BIA), mean (SD), kg	22.0 (7.9)	20.5 (7.9)	20.7 (7.4)	22.7 (6.7)	20.6 (7.1)
Total body fat (CT), mean (SD), cm^2	494.3 (167.0)	482.5 (166.5)	495.6 (173.7)	509.0 (130.0)	478.5 (151.5)
Visceral fat (CT), mean (SD), cm^2	171.9 (79.5)	174.8 (85.1)	187.5 (86.9)	178.6 (91.4)	174.1 (92.4)
Subcutaneous fat (CT), mean (SD), cm^2	257.3 (112.1)	241.0 (108.4)	238.6 (108.4)	261.1 (99.8)	237.1 (100.5)
Waist circumference (CT), mean (SD), cm	125.8 (14.0)	125.7 (13.4)	126.5 (14.2)	127.8 (11.8)	125.4 (13.3)
Waist circumference, mean (SD), cm	100.8 (12.1)	100.8 (11.2)	101.6 (11.5)	103.8 (9.2)	101.5 (9.9)

Missing values are 65 for BMI, 25 for midlife BMI, 57 for weight, 479 for maximum weight, 1666 for percent body fat and fat, 449 for the CT variables (total body fat, visceral fat, subcutaneous fat, and CT waist circumference), and 53 for waist circumference.

BIA, bioelectrical impedance analysis; SD, standard deviation.

*Proportion of cases that progressed from MGUS or LC-MGUS.

sample available for analysis, 1 subject had a missing consent form, and 1 subject had an M-protein concentration above the limit for MGUS and therefore fulfilling the criteria for smoldering MM. One subject was excluded from the progression analysis only because of zero follow-up days. Cases of MM and other LP diseases were found by cross-linking the AGES-RS data with the Icelandic Cancer Registry. The Icelandic Cancer Registry is in accordance with internationally accepted standards as it has high diagnostic accuracy and completeness (99%).[40] End of follow-up was March 2014.

Statistical analysis

Logistic regression was used to estimate the association between MGUS and LC-MGUS and the 11 obesity markers, and the results are presented as odds ratios (ORs) with 95% confidence intervals (95% CIs). Analyses were performed for both MGUS and LC-MGUS separately and together. Adjustment was made for age and sex in a multivariable analysis. Adjusting for height in multivariable models where height is not taken into account in the measurement, such as weight, waist circumference, and CT measures, did not affect the estimates and was therefore not included in the analysis. To test whether history of obesity had an effect on risk of MGUS/LC-MGUS, individuals were grouped in accordance to their midlife BMI and BMI at AGES-RS entry. Individuals with low midlife BMI and low BMI at AGES-RS entry were the referent group for groups with an increase in BMI, decrease in BMI, and constantly high BMI between these 2 time points. Cox proportional-hazard regression was used to test whether obesity was a risk factor for progression to MM or other LP diseases. Analyses were performed in R version 3.1.2.[41]

Results

A total of 5725 subjects were included in our analysis, with a mean age of 77 years (range 66-98). In the sample, 3306 (58%) individuals were women and 2419 were men. MGUS was identified in 300 (5.2%) subjects and LC-MGUS in 275 (4.8%) subjects (Table 1). Of the 300 MGUS patients, the Ig isotype was immunoglobulin G (IgG) in 159 (53%) patients, IgA in 27 (9%), IgM in 81 (27%), IgD in 1 (0.3%), and biclonal in 32 (10.7%). During a median follow-up of 8 years, a total of 18 individuals progressed to MM (17 of these from MGUS and 1 from LC-MGUS), and 11 individuals progressed to other LP diseases such as Hodgkin and non-Hodgkin lymphoma, Waldenström's macroglobulinemia, leukemia, chronic lymphocytic leukemia, and acute lymphocytic leukemia (2 of these from LC-MGUS).

No association was found between the 11 obesity markers and MGUS or LC-MGUS (Table 2). Additionally, BMI history was not

Table 2. ORs and 95% CIs for association between obesity and MGUS and LC-MGUS

	MGUS			LC-MGUS		
	n	OR	95% CI	n	OR	95% CI
Baseline BMI						
<25	101	Ref.	Ref.	93	Ref.	Ref.
25-29.9	146	1.14	0.88-1.49	121	1.07	0.81-1.41
≥30	49	0.81	0.57-1.16	58	1.14	0.81-1.61
Midlife BMI						
<25	139	Ref.	Ref.	128	Ref.	Ref.
≥25	159	1.15	0.90-1.45	146	1.10	0.86-1.41
Weight	296	0.97	0.91-1.05	272	1.04	0.96-1.12
Max weight	266	1.03	0.96-1.11	240	1.02	0.94-1.10
Percent body fat (BIA)	192	0.95	0.80-1.13	174	1.15	0.96-1.39
Fat (BIA)	192	0.94	0.76-1.16	174	1.10	0.88-1.36
Total body fat (CT)	269	1.00	0.99-1.00	242	1.00	1.00-1.01
Visceral fat (CT)	269	1.00	0.98-1.01	242	1.01	1.00-1.02
Subcutaneous fat (CT)	269	1.00	0.99-1.01	242	1.00	0.99-1.01
Waist circumference (CT)	269	1.00	0.94-1.06	242	1.05	0.99-1.13
Waist circumference	296	0.97	0.89-1.07	272	1.02	0.93-1.12

Results were obtained with multinomial logistic regression. ORs for continuous variables are per SD increase.

Ref., reference group.

associated with MGUS and LC-MGUS when joint effects of midlife BMI and BMI at study entry were examined, either when MGUS and LC-MGUS were analyzed together (Table 3) or separately (data not shown). Finally, based on a small number of cases, a nonsignificant increased risk of progression (hazard ratio [HR], 2.64; 95% CI, 0.93-7.48) from MGUS/LC-MGUS to MM was found with high midlife BMI (>25 kg/m^2). When other LP cases were combined with MM cases, the risk was statistically significantly increased (HR, 2.66; 95% CI, 1.17-6.05) (Table 4). There was no difference in distribution of known risk factors for MGUS progression (isotype, M-protein concentration, or FLC ratio) between individuals with low (<25 kg/m^2) and high (≥25 kg/m^2) BMI (data not shown).

Sensitivity analysis

Given the unusually high prevalence of LC-MGUS in our cohort (4.8%), with a high prevalence of κ cases (96%), we performed an additional sensitivity analysis. As the distribution of log-transformed κ and λ values resembled the normal distribution, we evaluated the effect of using the 97.5th percentile as a cutoff for normal values for the involved light-chain. Using a definition of LC-MGUS as a pathological FLC ratio of <0.26 and >1.65, in combination with an increased concentration of >40.0 mg/L of the light-chain involved, resulted in 52 LC-MGUS cases (0.9%), of which 41 were κ and 11 were λ. Results from the sensitivity analysis confirmed previous findings (data not shown).

Discussion

In this large population-based screening study on 5725 subjects, we found that obesity is not associated with MGUS or LC-MGUS. This was true independent of various obesity assessment techniques, including BIA, CT scans, and other anthropometric measurements such as height, weight, and waist circumference.

Table 3. Joint effect of midlife BMI and baseline BMI on MGUS/LC-MGUS

Joint BMI categories	MGUS/LC-MGUS, n (%)	OR	95% CI
Low midlife BMI, low current BMI	149 (27.8)	Ref.	Ref.
Low midlife BMI, medium current BMI	99 (21.0)	0.99	0.98-1.01
Low midlife BMI, high current BMI	16 (3.8)	1.00	0.97-1.04
High midlife BMI, low current BMI	45 (5.9)	1.03	1.00-1.04
High midlife BMI, medium current BMI	166 (22.6)	1.02	1.00-1.04
High midlife BMI, high current BMI	91 (19.0)	0.99	0.98-1.01

Results were obtained with logistic regression. Low midlife BMI, <25 kg/m^2; high midlife BMI, ≥25 kg/m^2; low current BMI, <25 kg/m^2; medium current BMI, 25-29.9 kg/m^2; high current BMI, ≥30 kg/m^2.

Additionally, we did not find history of obesity or weight change to have an effect on MGUS/LC-MGUS. Interestingly, we found that high BMI, measured at midlife, was associated with an increased risk of progression to MM and other LP diseases.

We found no association between any of the 11 obesity measures and MGUS or LC-MGUS. Previously, only 2 studies have examined the association between MGUS and obesity, with conflicting results.[32,33] Our results are in line with the population-based National Health and Nutritional Examination Survey study (N = 12 482; MGUS cases, 365) that found that BMI did not have an effect on MGUS risk,[33] whereas the Southern Community study (N = 1996; MGUS cases, 60) found a twofold increased risk of MGUS in obese (BMI >30 kg/m^2) vs nonobese women.[32] Differences in the nature of the cohorts such as the cohort size, and age, sex, and race distribution, as well as differences in recruitment of participants, might explain the discrepancy between the studies. It has been suggested that the increased risk of MM with increasing BMI[22,23] is explained by an increased risk of MGUS, which in turn puts individuals at risk for developing MM.[32] Our findings do not confirm this. LC-MGUS is a newly defined disorder, and factors that influence the development of the condition and its progression are largely unknown. This is the first study to date to examine the association with obesity.

Our results suggest that high BMI during midlife is associated with an increased risk of progression from MGUS/LC-MGUS to MM and other LP diseases later in life. This was not explained by known risk factors for progression. Previous studies have indicated that obesity might have a role in the etiology of MM.[21,22,25,26] Recent pooled analysis of 20 prospective studies found that waist circumference is a risk factor for MM mortality and that BMI in early adulthood plays an important role in myelomagenesis.[25] Additionally, on the basis of the available data, WHO recently concluded that there now is sufficient evidence behind the association between body fatness and MM.[6] To date, only 1 study has examined the role of obesity in the progression of MGUS to MM. A study on a cohort of US veterans within the Veterans Health Administration system found an increased risk of MM to be associated with both overweight (HR, 1.55; 95% CI, 1.16-2.06) and obesity (HR, 1.98; 95% CI, 1.47-2.68) at MGUS diagnosis.[42] We thus speculate that the observed risk of MM in individuals with obesity is not because of increased risk of MGUS, but rather that they have similar MGUS prevalence but a higher risk of progression.

Although our results are limited by the small number of cases and should be interpreted with caution, the consistently high risk

Table 4. HRs and 95% CIs for obesity and risk of progression from MGUS to MM and other LP diseases

	MM			MM and other LP diseases		
	n	HR	95% CI	n	HR	95% CI
Baseline BMI						
<25.0	5	Ref.	Ref.	8	Ref.	Ref.
≥25.0	13	1.33	0.47-3.75	21	1.30	0.57-2.94
Midlife BMI						
<25.0	5	Ref.	Ref.	8	Ref.	Ref.
≥25.0	13	2.64	0.93-7.48	21	2.66	1.17-6.05
Weight	18	1.10	0.84-1.44	29	1.04	0.84-1.29
Max weight	18	1.16	0.88-1.54	29	1.15	0.93-1.43
Percent body fat (BIA)	10	1.54	0.35-6.86	19	0.96	0.88-1.05
Fat (BIA)	10	1.92	0.81-4.57	19	0.79	0.29-2.13
Total body fat (CT)	17	1.00	0.98-1.02	28	1.00	0.98-1.01
Visceral fat (CT)	17	1.00	0.96-1.05	28	0.99	0.95-1.03
Subcutaneous fat (CT)	17	1.01	0.97-1.04	28	0.99	0.96-1.02
Waist circumference (CT)	17	1.01	0.97-1.04	28	0.96	0.79-1.16
Waist circumference	18	1.16	0.82-1.64	29	1.00	0.75-1.33

Results were obtained with Cox proportional hazard regression. HRs for continuous variables are per SD increase.

estimate both when MM was analyzed with and without other LP diseases in our main analysis and sensitivity analysis, and a recent study on the role of obesity in MGUS progression,[42] strengthens the notion that obesity plays a role in myelomagenesis and is possibly the first identified modifiable risk factor for progression of MGUS to MM. We did not find body weight or body composition at study baseline in MGUS/LC-MGUS cases to be risk factors for progression to MM and other LP diseases. The induction time for MM and other LP diseases is long, and we do not have information on how long each individual has met the criteria for MGUS/LC-MGUS before developing MM or other LP diseases. A US-based screening study on a clinical cohort concluded that when first clinically recognized, MGUS has likely been present in an undetected state for a median duration of >10 years,[43] and the interval from diagnosis of MGUS to diagnosis of MM or related diseases ranges from 1 to 32 years (median, 10.4 years) according to a follow-up study on 241 MGUS patients at Mayo Clinic.[44] Based on these results and the high mean age of the population under study (77 years), it is possible that body weight and composition at time of MGUS/LC-MGUS diagnosis is not a reliable indicator for MM progression, as earlier life physique might be.

A major strength of this study is the design as this is a population-based screened cohort study with high internal and external validity. Another major strength is the use of various and precise measures to assess obesity. BMI is a limited measure as it does not give information on fat distribution and does not distinguish between lean and fat mass; however, studies have shown that BMI is highly correlated with other more precise measures of body composition and fat distribution and has been shown to be at least approximately equivalent in the ability to predict diseases.[45] We found in our data that the obesity measures were highly correlated (r = 0.37-0.95), but the lack of an acceptable gold standard for measuring body fatness makes it difficult to use only 1 measure. Using multiple markers for late-life body fatness deals with the limitations that pertain to each measure and strengthens our findings. An additional strength is the consistency in the findings throughout the assessment methods.

Some limitations need to be kept in mind when interpreting the results. Although this is a screened study, a selection bias might be present. The mean age is high (77 years), and therefore, the cohort might represent a selection of the population that is healthier than the general population. Additionally, Iceland has an exclusively white population, and in view of previous findings regarding MGUS variance across ethnic groups,[18,38] our results may not be applicable to other races. As we do not have bone marrow samples from our participants, we cannot therefore truly distinguish what could be considered as smoldering MM. Missing data highly influence the number of cases in our BIA models, mainly in our progression analysis; these results should therefore be interpreted with caution. A low number of cases that progressed could mean that we had insufficient statistical power, which might affect our results.

In conclusion, in this Icelandic population-based cohort study, obesity is not associated with MGUS or LC-MGUS. However, based on 29 cases that progressed to MM or other LP diseases later in life, our study found midlife obesity to be a risk factor for progression among individuals diagnosed with MGUS/LC-MGUS. This study provides evidence that obesity might be the first modifiable risk factor for MGUS/LC-MGUS progression, but more studies, both large-scale population-based studies and clinical trials, are needed for better understanding of the etiology of MGUS/LC-MGUS and MM.

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Authorship

Contribution: S.Y.K., E.K.L., O.L., and M.T. designed the study; R.C. and D.B. performed laboratory analysis; M.T. and S.H.L. performed the statistical analysis; M.T. and S.Y.K. wrote the manuscript; and all authors were involved in the interpretation of the results and the preparation of the final manuscript.

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Correspondence: Sigurdur Y. Kristinsson, Faculty of Medicine, University of Iceland, Reykjavik, Iceland; e-mail: sigyngvi@hi.is.

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Paper II

PLOS ONE

RESEARCH ARTICLE

Dietary intake is associated with risk of multiple myeloma and its precursor disease

Marianna Thordardottir[1]*, Ebba K. Lindqvist[2], Sigrun H. Lund[3], Rene Costello[4], Debra Burton[4], Laufey Steingrimsdottir[5], Neha Korde[6], Sham Mailankody[6], Gudny Eiriksdottir[7], Lenore J. Launer[8], Vilmundur Gudnason[1,7], Tamara B. Harris[8], Ola Landgren[6], Johanna E. Torfadottir[3,9☯], Sigurdur Y. Kristinsson[1,2☯]

1 Faculty of Medicine, University of Iceland, Reykjavik, Iceland, **2** Department of Medicine, Division of Hematology, Karolinska University Hospital and Karolinska Institutet, Stockholm, Sweden, **3** Centre of Public Health Sciences, Faculty of Medicine, University of Iceland, Reykjavik, Iceland, **4** Multiple Myeloma Section, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, United States of America, **5** Faculty of Food Science and Human Nutrition, University of Iceland, Reykjavik, Iceland, **6** Myeloma Service, Division of Hematologic Oncology, Memorial Sloan-Kettering Cancer Center, New York, New York, United States of America, **7** The Icelandic Heart Association, Kopavogur, Iceland, **8** Laboratory of Epidemiology and Population Sciences, Intramural Research Program, National Institute on Aging, Bethesda, Maryland, United States of America, **9** The Icelandic Cancer Society, Reykjavik, Iceland

☯ These authors contributed equally to this work.
* mthordar@hi.is

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Data Availability Statement: Data used in this study were obtained through collaboration between the University of Iceland, the Icelandic Heart Association (IHA), and the Icelandic Cancer Registry (ICR). Data from the IHA cannot be made publicly available since the informed consent signed by the participants, prohibits data sharing on an individual level as the approval for the study by the Icelandic National Bioethics Committee is based on. Secondly, data from the ICR is protected by Icelandic data protection laws which forbids data being publicly available. Requests for the data

Abstract

The etiology of monoclonal gammopathy of undetermined significance (MGUS), the precursor state of multiple myeloma (MM), is mostly unknown and no studies have been conducted on the effect of diet on MGUS or progression from MGUS to MM. We aimed to explore the association between common foods and MGUS and progression to MM. Data from the population-based AGES Study (N = 5,764) were utilized. Food frequency questionnaire was used to assess dietary intake during adolescence, midlife, and late life. Serum protein electrophoresis and serum free light-chain assay was performed to identify MGUS (n = 300) and LC-MGUS cases (n = 275). We cross linked our data with the Icelandic Cancer Registry to find cases of MM in the study group. We found that intake of fruit at least three times per week during adolescence was associated with lower risk of MGUS when compared to lower fruit consumption (OR = 0.62, 95% CI 0.41–0.95). We additionally found that intake of fruit at least three times per week during the late life period was associated with decreased risk of progressing from MGUS to MM (HR = 0.34, 95% CI 0.13–0.89) when compared to lower intake. Adolescent intake of fruit may reduce risk of MGUS, whereas fruit intake after MGUS onset may reduce risk of progressing to MM. Our findings suggest that diet might alter the risk of developing MGUS and progression to MM.

Introduction

All cases of the plasma cell malignancy multiple myeloma (MM) are preceded by monoclonal gammopathy of undetermined significance (MGUS) [1, 2], a premalignant asymptomatic

may be sent to the AGES Reykjavik Study Executive Committee, contact: AGES_data_request@hjarta.is. and the ICR (contact: Laufey Tryggvadottir, laufeyt@krabb.is) with approval from the Icelandic National Bioethics Committee (https://www.vsn.is/en/content/bioethics-committee-system, vsn@vsn.is).

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condition characterized by the presence of an M-protein in serum or by abnormal ratio between the free light-chains kappa and lambda (light-chain MGUS), without indication of MM or other lymphoproliferative (LP) diseases [3–5]. The prevalence of MGUS is approximately 5% in those older than 70 years and increases with age [6]. It is estimated that average risk of progression from MGUS to MM is approximately 1% per year [7, 8]. Light-chain MGUS (LC-MGUS) has been described as a precursor to light-chain MM, with a prevalence of 0.7–0.8% [4, 5].

As previously reported, the etiology of MGUS and LC-MGUS is mostly unknown [9]. However, studies have reported a higher risk of MGUS among males [6], black race [10, 11], in individuals with family history of MGUS and related diseases [12], in individuals with previous personal or family history of immune-related conditions [13, 14], and recently in those who have been largely exposed to Agent Orange, an herbicide and defoliant chemical [15]. The literature on the etiology of MM is more extensive. An elevated risk of MM has been found to be associated with low occupation-based socioeconomic status, income, education [16], and high body mass index (BMI) [9, 17, 18]. The International Agency for Research on Cancer has recently concluded, that there is now adequate evidence behind the association between body weight and MM [19]. We have recently shown that high BMI, measured during midlife (≈50 years old), was associated with an increased risk of progressing from MGUS to MM and other LP diseases later in life, suggesting that exposures that originate during the midlife period, and perhaps earlier in life, play a role in the pathogenesis of MM and related diseases diagnosed later in life [9]. Since obesity has various underlying causes this indicates that lifestyle-related factors, such as diet, are important risk factors for MM. However, epidemiological evidence on the effect of diet on MM is scarce and the results are inconclusive [20–24]. The strongest evidence exists for fish intake, where inverse association has been reported in few case-control studies [21, 24–26], two of which reported dose-response relationship [21, 25]. No studies, to our knowledge, on the association between diet and MGUS or LC-MGUS have been conducted. Additionally, MGUS has been detected in individuals as young as 10–19 years old [27], emphasizing the importance of studying the effect of early and midlife exposures on MGUS. Many of the residential regions in Iceland were relatively isolated during the first half of the 20th century. Due to that, there was a considerable variation in diet across the country. The inhabitants' diets was largely limited to locally produced food available on side such as fish in sea villages and livestock at the farm [28]. Availability of fruit and vegetables was limited due to unfavorable weather conditions and limited import. Iceland is therefore an ideal forum for dietary research due to extremes in intake of common food groups. During the second half of the 20th century the diet became more westernized [29].

As all MM cases first go through the MGUS stage it is important to disentangle these associations with the objective to identify separate risk factors for MGUS, LC-MGUS, and progression to MM. The overarching aim of this study was therefore to analyze the association between diet throughout the lifespan and risk of MGUS and LC-MGUS using population-based data. Secondly, we aimed to analyze whether diet was a risk factor for progression to MM and other LP diseases in individuals with MGUS or LC-MGUS.

Materials and methods

Study population

For this study we used data from the Age, Gene/Environment Susceptibility–Reykjavik Study (AGES Study). The AGES Study is a continuation of the population-based Reykjavik Study, initiated in 1967 when all residents, born 1907–1935, of the Reykjavik metropolitan area were invited to participate in a prospective cohort study with the objective of examining risk factors

for cardiovascular diseases. A total of 71% (n = 19,350) of the invited residents consented to participate during the years of 1967–1996 [30–32]. Of the 11,549 Reykjavik Study cohort members still alive in March 2002, when the AGES Study was initiated, 8,030 individuals were randomly chosen to take part in the study. By 2006, when the study ended, 5,764 (71.8%) had participated. Detailed description of the study and collection of data has been previously published [32], however in short, data were collected during three separate examinations using standardized protocols. The first visit included e.g. blood draw, anthropometry, electrocardiography, and extensive questionnaire including e.g. health history, lifestyle practices, and food history. The second examination included imaging protocols and the third examination included e.g. dementia assessments and vision screening [32].

At study entry the participants signed an informed consent form. The study was approved by the Icelandic Data Protection Authority, the Icelandic National Bioethics Committee (VSN-00-063-V35), and the Institutional Review Board of the National Institute on Aging in the USA.

Dietary habits across the lifespan

At AGES Study entry the participants provided retrospective information on dietary habits during adolescence (14–19 years old) and midlife (40–50 years old), as well as information on current dietary habits using a food frequency questionnaire. The questionnaire included a total of 63 questions (16 from the adolescent period, 17 from the midlife period, and 30 from the late life period) regarding intake of common foods and food groups i.e. total fish intake (and additional question on salted or smoked fish), fish oil, total meat intake (and additional question on salted or smoked meat), milk and milk products, fruit, vegetables (excluding potatoes), rye bread and flatbread, blood or liver sausage, oatmeal and muesli, potatoes, and whole wheat bread. Only foods and food groups that were included in the questionnaire from all three life stages were used for this study, except for whole wheat bread (midlife and late life only). The participants reported frequency of intake in each time period using the following response categories for meat, milk and milk products, fruit, vegetables, rye bread and flatbread, blood or liver sausage, oatmeal and muesli, potatoes, and whole wheat bread: *never*, *less than once a week*, *1–2 times a week*, *3–4 times a week*, *5–6 times a week*, *daily*, and *more than once a day*. For fish oil the categories were the same except for: *more than once a day*. The categories for salted and smoked meat and fish were: *never*, *less than once a month*, *1–3 times a month*, *1–2 times a week*, *3–6 times a week*, and *daily or more often*. The food frequency questionnaire included three questions on fish consumption; frequency of consumption of fish in salad or as topping on bread, fish as main meal (including salted or smoked fish), and consumption of salted or smoked fish separately. Weekly intake of fish meals and fish in salad or as topping was combined into one variable and the daily total intake was converted into total fish portions per week, as has been previously described [33]. The validation study of the midlife diet took advantage of available data from a detailed nutrition study performed 18 years previously, using dietary history and the present diet was validated using a 3-day dietary history as a reference method. The AGES food frequency questionnaire was found suitable to rank individuals by their intake for most food groups from the midlife and late period [34, 35].

Ascertainment of outcomes

As previously described [9, 36], a conventional agarose-gel serum protein electrophoresis (SPEP) was performed in 2013–2014 on all subjects from the AGES Study cohort to identify MGUS cases. A 0.5 mL serum sample, collected at study entry (2002–2006), was obtained for each study subject and samples with an equivocal or definite M-protein present on SPEP were

then subjected to serum protein immunofixation for conformation and typing of the M-protein [6]. Serum free light chain (FLC) assay was performed on all samples [37]. The sensitivity and specificity of the laboratory tests have been previously published [38]. All testing was done by individuals blinded to all demographics and other details related to the samples.

MGUS cases were defined as having M-protein bands (detectable on SPEP or immunofixation) and an elevated M-protein concentration ($\leq$30g/L) [39]. LC-MGUS cases were defined as having no visible M-protein, a pathological FLC ratio ($<$0.26 or $>$1.65) on FLC analysis, and an increased free light-chain concentration (f-kappa $>$19.4 mg/L, f-lambda $>$26.3 mg/L) [40]. We ascertained MM diagnosis and diagnosis of other LP diseases through linkage with the nationwide Icelandic Cancer Registry [41]. The start date of follow-up was at AGES Study entry (March 2002-February 2006) until March 2014.

Exclusion from analysis

For this study we excluded a total of 40 (0.7%) subjects from analysis, 21 due to previous LP diseases, 16 due to missing blood sample, one due to absent consent form, and one subject had high M-protein concentration at baseline and therefore fulfilled criteria for smoldering MM. One subject was additionally excluded from the progression analysis due to lack of follow-up. Our analyses include individuals responding to the dietary questions, ranging from 5,270 to 5,304 from the adolescent period, from 5,279 to 5,301 from the midlife period, and from 507–511 from the late life period, depending on the question.

Statistical analysis

A total of 12 food items were analyzed for the adolescent period and 13 for both midlife and the late life period. Logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (95% CI) for MGUS and LC-MGUS according to adolescent and midlife diet. For each type of food or food group, participants were grouped according to their frequency of intake or portions per week (total fish consumption only). Each type of food or food group was analyzed in an age and sex adjusted model (Model 1) and additionally in a fully adjusted model where all the foods and food groups were simultaneously added to one model, including age and sex (Model 2). An additional adjustment was made for physical activity and BMI measured in midlife in the adolescent and midlife models, and since the adjustment did not affect our results physical activity and BMI were omitted from the models. Additionally, we cross-classified the intake from the adolescent and midlife periods by combining individuals with low adolescent intake and low midlife intake for each type of food and set them as a reference group for low and high, high and low, and high and high intake at these time periods. We then used logistic regression to test the association between the cross-classified categories of intake and MGUS and LC-MGUS combined. Models were adjusted for age and sex.

Cox proportional hazard regression was used to test whether diet at study entry was a associated with progression from MGUS/LC-MGUS to MM or other LP diseases. For this analysis only individuals with MGUS and LC-MGUS were used, and due to few cases of MM and other LP disease they were not analyzed separately. Results are presented as hazard ratios (HR) with 95% CI. All models were adjusted for sex and age at AGES Study entry. We further adjusted our models for BMI and physical activity and we tested the association in a model where all the foods were simultaneously added to one model. Due to few number of cases and therefore low statistical power, and no effect on results, these analyses were not included in our results. All analyses were performed in R version 3.3.3.[42]

Sensitivity analysis

As described in detail previously [9, 36], we had an unusually high prevalence of LC-MGUS in our cohort (4.8%), mainly due to a high prevalence of kappa cases (96%). The distribution of log-transformed kappa and lambda values was found to resembled the normal distribution, and therefore the cut-off for the involved chains was moved to the 97.5th percentile. A definition of LC-MGUS as a pathological FLC-ratio of < 0.26 and > 1.65, in combination with an increased concentration of more than 40.0 mg/L of the light-chain involved was used to perform sensitivity analysis on the association between diet and MGUS/LC-MGUS.

Results

The mean age of participants was 77 years (range 66–98) at study baseline. MGUS and LC-MGUS was identified in 300 (5.2%) and 275 (4.8%) subjects, respectively (Table 1). Using the modified definition of LC-MGUS resulted in 52 cases. By cross linking the AGES Study cohort to the Icelandic Cancer Registry we found that 18 participants progressed to MM, of which one from LC-MGUS during a median follow-up of 8 years. Additionally, 11 progressed to other LP diseases (Hodgkin's, Non-Hodgkin's lymphoma, Waldenström's macroglobulinemia, lymphoid leukemia, chronic lymphocytic leukemia, and acute lymphocytic leukemia), of which two from LC-MGUS.

Adolescent intake

We found, in Model 2, that intake of fruit at least three times per week during the adolescent period was associated with lower risk of MGUS when compared intake less than three times per week (OR = 0.62, 95% CI 0.41–0.95). Consumption of other food items during that period was not associated with MGUS (Table 2). Additionally, we found that intake of fish at least two times per week was associated with higher risk of LC-MGUS (OR = 1.33, 95% CI 1.02–1.73) when compared to intake less than two times per week. Adolescent consumption of other foods was not associated with LC-MGUS (Table 2). In our sensitivity analysis we did not find any association between fish intake and risk of LC-MGUS in Model 2 as we did in our primary analysis. No association was found between consumption of other food items and LC-MGUS in our sensitivity analysis.

Midlife intake

We found in Model 2 that intake of whole wheat bread at least five times per week was associated with lower risk of MGUS (OR = 0.75, 95% CI 0.57–0.99) when compared to participants

Table 1. Characteristics of study participants at AGES study entry.

	Without MGUS	MGUS	LC-MGUS	MM	LP
	n = 5,150 (90.0%)	n = 300 (5.2%)	n = 275 (4.8%)	n = 18 (3.1%)*	n = 11 (5.1%)*
Gender, n. (%)					
Female	3,046 (59.1)	141 (47.0)	119 (43.3)	10 (55.6)	13 (46.4)
Male	2,104 (40.9)	159 (53.0)	156 (56.7)	8 (44.4)	15 (53.6)
Mean age, years (range)	76.8 (66–98)	78.3 (67–93)	79.4 (66–97)	77.8 (69–87)	77.5 (68–87)
BMI, kg/m^2 (mean)	27.0	26.7	27.0	27.2	26.5
BMI midlife, kg/m^2 (mean)	25.2	25.5	25.6	26.2	26.2

*Proportion of cases that progressed from MGUS or LC-MGUS.

Abbreviations: MGUS—Monoclonal gammopathy of undetermined significance, LC-MGUS—Light chain monoclonal gammopathy of undetermined significance, MM—Multiple myeloma, LP—Other lymphoproliferative diseases.

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Table 2. The association between diet in adolescence and MGUS and LC-MGUS.

				Model 1a				Model 2b			
	No MGUS	MGUS	LC-MGUS	MGUS		LC-MGUS		MGUS		LC-MGUS	
	n (%)	n (%)	n (%)	OR	95%CI	OR	95% CI	OR	95%CI	OR	95% CI
Fish											
≤ 2 portions p/w	2,335 (49.1)	140 (52.2)	99 (40.7)	1.00		1.00		1.00		1.00	
> 2 portions p/w	2,424 (50.9)	128 (47.8)	144 (59.3)	0.85	0.66–1.09	1.33	1.02–1.73	0.88	0.68–1.14	1.39	1.06–1.83
Fish oil											
less than weekly	2,280 (47.6)	119 (44.4)	128 (52.7)	1.00		1.00		1.00		1.00	
weekly or more	2,509 (52.4)	149 (55.6)	115 (47.3)	1.13	0.88–1.45	0.82	0.63–1.06	1.14	0.89–1.48	0.87	0.66–1.14
Salted fish											
3 times a month or less	2,210 (46.4)	128 (47.8)	114 (46.9)	1.00		1.00		1.00		1.00	
once p/w or more	2,555 (53.6)	140 (52.2)	129 (53.1)	0.85	0.66–1.10	0.84	0.64–1.09	0.95	0.71–1.26	0.89	0.66–1.21
Meat											
2 times p/w or less	1,689 (35.3)	103 (38.6)	91 (37.6)	1.00		1.00		1.00		1.00	
3 times p/w or more	3,093 (64.7)	164 (61.4)	151 (62.4)	0.87	0.67–1.12	0.89	0.68–1.17	0.89	0.68–1.15	0.86	0.62–1.14
Smoked/salted meat											
3 times a month or less	3,111 (65.2)	184 (68.7)	157 (64.9)	1.00		1.00		1.00		1.00	
Once a week or more	1,663 (34.8)	84 (31.3)	85 (35.1)	0.79	0.60–1.03	0.90	0.68–1.18	0.82	0.61–1.10	0.94	0.69–1.28
Milk and milk products											
less than daily	1,074 (22.4)	65 (24.3)	63 (25.9)	1.00		1.00		1.00		1.00	
daily	3,719 (77.6)	203 (75.7)	180 (74.1)	0.87	0.65–1.17	0.79	0.59–1.07	0.91	0.67–1.24	0.85	0.62–1.17
Fruit											
2 times p/w or less	4,077 (85.3)	241 (89.9)	217 (89.7)	1.00		1.00		1.00		1.00	
3 times p/w or more	701 (14.7)	27 (10.1)	25 (10.3)	0.71	0.47–1.06	0.75	0.49–1.15	0.62	0.41–0.95	0.81	0.52–1.26
Vegetables											
2 times p/w or less	3,517 (73.5)	197 (73.8)	197 (81.1)	1.00		1.00		1.00		1.00	
3 times p/w or more	1,265 (26.5)	70 (26.2)	46 (18.9)	1.06	0.80–1.41	0.71	0.51–0.99	1.17	0.87–1.57	0.78	0.55–1.10
Rye bread/flatbread											
less than daily	2,481 (52.0)	141 (52.6)	120 (49.4)	1.00		1.00		1.00		1.00	
daily	2,289 (48.0)	127 (47.4)	123 (50.6)	0.88	0.68–1.14	0.94	0.72–1.22	0.94	0.71–1.23	1.00	0.75–1.34
Sausage/liver											
less than weekly	1,252 (26.1)	74 (27.6)	68 (28.0)	1.00		1.00		1.00		1.00	
weekly or more	3,539 (73.9)	194 (72.4)	175 (72.0)	0.84	0.63–1.11	0.78	0.58–1.04	0.86	0.65–1.18	0.85	0.61–1.18
Oatmeal/muesli											
2 times p/w or less	2,119 (44.4)	110 (41.4)	108 (44.6)	1.00		1.00		1.00		1.00	
3 times p/w or more	2,650 (55.7)	156 (58.6)	134 (55.4)	1.02	0.79–1.31	0.84	0.64–1.09	1.09	0.83–1.44	0.93	0.70–1.24
Potatoes											
less than daily	483 (10.1)	35 (13.1)	25 (10.3)	1.00		1.00		1.00		1.00	
daily	4,300 (89.9)	233 (86.9)	217 (89.7)	0.76	0.52–1.10	0.99	0.64–1.51	0.81	0.55–1.20	1.04	0.67–1.63

[a]Adjusted for age and sex.

[b]Adjusted for age, sex and the other food groups.

Abbreviations: MGUS—Monoclonal gammopathy of undetermined significance, LC-MGUS—Light chain monoclonal gammopathy of undetermined significance, OR—Odds ratio, CI—confidence interval

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with intake of less than five times per week. Intake of other foods from midlife was not associated with MGUS (Table 3). Midlife intake of the tested food items was not associated with LC-MGUS (Table 3). In our sensitivity analysis we found in Model 2 that midlife intake of

Table 3. The association between midlife diet and MGUS and LC-MGUS.

				Model 1a				Model 2b			
	No MGUS	MGUS	LC-MGUS	MGUS		LC-MGUS		MGUS		LC-MGUS	
	n (%)	n (%)	n (%)	OR	95%CI	OR	95% CI	OR	95%CI	OR	95% CI
Fish											
≤ 2 portions p/w	565 (11.8)	35 (13.1)	33 (13.6)	1.00		1.00		1.00		1.00	
> 2 portions p/w	4,204 (88.2)	233 (86.9)	209 (86.4)	0.84	0.58–1.22	0.77	0.52–1.13	0.87	0.59–1.28	0.80	0.54–1.19
Fish oil											
less than weekly	1,852 (38.7)	97 (36.3)	97 (39.9)	1.00		1.00		1.00		1.00	
weekly or more	2,931 (61.3)	170 (63.7)	146 (60.1)	1.09	0.84–1.41	0.94	0.72–1.23	1.10	0.84–1.44	0.95	0.72–1.25
Salted fish											
3 times a month or less	3,241 (67.8)	187 (69.8)	161 (66.3)	1.00		1.00		1.00		1.00	
once p/w or more	1,541 (32.2)	81 (30.2)	82 (33.7)	0.81	0.62–1.07	0.91	0.67–1.20	0.82	0.60–1.13	0.90	0.65–1.25
Meat											
2 times p/w or less	1,809 (37.8)	106 (39.6)	93 (38.3)	1.00		1.00		1.00		1.00	
3 times p/w or more	2,977 (62.2)	162 (60.4)	150 (61.7)	0.94	0.73–1.21	1.03	0.78–1.35	0.93	0.72–1.21	1.00	0.76–1.32
Smoked/salted meat											
3 times a month or less	3,530 (73.8)	199 (74.3)	170 (70.2)	1.00		1.00		1.00		1.00	
Once a week or more	1,252 (26.2)	69 (25.7)	72 (29.8)	0.89	0.67–1.18	1.05	0.79–1.40	0.95	0.68–1.32	1.12	0.80–1.56
Milk and milk products											
less than daily	1,957 (40.9)	99 (37.1)	90 (37.2)	1.00		1.00		1.00		1.00	
daily	2,823 (59.1)	168 (62.9)	152 (62.8)	1.11	0.86–1.43	1.08	0.82–1.41	1.17	0.89–1.53	1.15	0.87–1.53
Fruit											
2 times p/w or less	3,271 (68.4)	185 (69.0)	181 (74.5)	1.00		1.00		1.00		1.00	
3 times p/w or more	1,512 (31.6)	83 (31.0)	62 (25.5)	1.10	0.84–1.44	0.88	0.65–1.19	1.19	0.87–1.62	0.84	0.60–1.18
Vegetables											
2 times p/w or less	3,081 (64.6)	182 (68.2)	162 (66.7)	1.00		1.00		1.00		1.00	
3 times p/w or more	1,692 (35.4)	85 (31.8)	81 (33.3)	0.92	0.70–1.20	1.02	0.77–1.34	0.84	0.62–1.14	1.11	0.81–1.52
Rye bread/flatbread											
less than daily	3,264 (68.1)	185 (69.0)	166 (68.3)	1.00		1.00		1.00		1.00	
daily	1,526 (31.9)	83 (31.0)	77 (31.7)	0.86	0.66–1.13	0.83	0.62-.1.10	0.91	0.68–1.22	0.84	0.62–1.15
Sausage/liver											
less than weekly	2,322 (48.5)	123 (45.9)	112 (46.1)	1.00		1.00		1.00		1.00	
weekly or more	2,466 (51.5)	145 (54.1)	131 (53.9)	1.05	0.82–1.35	1.00	0.77–1.30	1.07	0.82–1.39	1.00	0.76–1.33
Oatmeal/muesli											
2 times p/w or less	2,910 (60.9)	147 (55.1)	140 (57.9)	1.00		1.00		1.00		1.00	
3 times p/w or more	1,869 (39.1)	120 (44.9)	102 (42.1)	1.18	0.92–1.52	1.01	0.77–1.31	1.27	0.94–1.60	1.05	0.80–1.40
Potatoes											
less than daily	742 (15.5)	48 (17.9)	40 (16.5)	1.00		1.00		1.00		1.00	
daily	4,046 (84.5)	220 (82.1)	203 (83.5)	0.80	0.58–1.11	0.86	0.60–1.22	0.82	0.58–1.16	0.88	0.61–1.28
Whole wheat bread											
4 times p/w or less	1,489 (31.2)	101 (38.1)	87 (36.1)	1.00		1.00		1.00		1.00	
5 times p/w or more	3,291 (68.8)	164 (61.9)	154 (63.9)	0.76	0.59–0.98	0.83	0.64–1.09	0.75	0.57–0.99	0.86	0.64–1.15

[a]Adjusted for age and sex.

[b]Adjusted for age, sex and the other food groups.

Abbreviations: MGUS—Monoclonal gammopathy of undetermined significance, LC-MGUS—Light chain monoclonal gammopathy of undetermined significance, OR—Odds ratio, CI—confidence interval.

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meat at least three times per week and daily intake of rye bread and flatbread was associated with lower risk of LC-MGUS (OR = 0.44, 95% CI 0.23–0.84 and OR = 0.32, 95% CI 0.14–0.78, respectively) when compared to lower intake. No association was found between consumption of other food items from the midlife periods and LC-MGUS in our sensitivity analysis.

Cross-classification across two time points

Looking at both the adolescent and midlife period together, no association was found between any of the food items and MGUS/LC-MGUS (S1 Table). However, in our sensitivity analysis (S2 Table) we found that daily intake of rye bread and flatbread and potatoes during both the adolescent and midlife periods was associated with lower risk of MGUS/LC-MGUS when compared to less than daily intake at both periods (OR = 0.70, 95% CI 0.55–0.95 and OR = 0.63, 95% CI 0.45–0.96, respectively).

Progression to multiple myeloma and other lymphoproliferative diseases

We found, in a sex and age adjusted model, that fruit intake at least three times per week was inversely associated with risk of progression to MM (HR = 0.34, 95% CI 0.13–0.89) when compared to intake less than three times per week. The association remained statistically significant when cases of other LP diseases were combined with MM cases (HR = 0.45, 95% CI 0.21–0.96). Intake of other food items was not associated with risk of progression to MM and other LP diseases (Table 4). Similar results were found when analyzing risk of progression in MGUS cases only.

Discussion

In this population-based study we found, using a food frequency questionnaire for evaluation of dietary intake during three separate time periods, that food intake may affect risk of MGUS, and specifically that intake of fruit at least three times per week during adolescence and midlife intake of whole wheat bread at least five times per week, was associated with lower risk of MGUS when compared to lower intakes. Additionally, we found, albeit based on few cases, that late life intake of fruit at least three times per week in individuals with MGUS or LC-MGUS was associated with lower risk of progressing to MM and other LP diseases. Adjusting for BMI and physical activity did not change the results. These findings suggest that dietary habits might influence the etiology of MGUS and progression to MM and other LP diseases.

We found that intake of fruit at least three times per week during the adolescent period was inversely associated with risk of MGUS later in life when compared to lower intake, and, based on a small number of cases, we found that late life intake of fruit at least three times per week in patients with MGUS or LC-MGUS was associated with lower risk of progression. The findings are in accordance with other studies consistently showing high intake of fruit and other plant foods to be inversely associated with different types of cancer [43, 44]. A review of findings from the European Prospective Investigation into Cancer and Nutrition (EPIC) from 2014 reported an inverse association between fruit intake and cancer risk at some sites [43]. The literature regarding fruit and risk of MM is scarce. To date, only two studies have examined the role of fruit in the etiology of MM. Both are US-based case-control studies on adults already diagnosed with MM, with no association reported [21, 23]. Major differences in the amount (intake levels) and time of exposure (period of life) and time of assessment (exposure assessed after MM diagnosis) could explain the discrepancy with regards to our study. A potential biologic mechanism for our finding is perhaps the anti-carcinogenic effect of vitamin C as it traps free radicals and protects against oxidation [45]. Nevertheless, very few participants reported daily or more than daily fruit intake in present study. Therefore, the cut-off for

Table 4. The association between late life diet and risk of progression from MGUS and LC-MGUS to multiple myeloma and other lymphoproliferative diseases.

	Total	Multiple myeloma	Multiple myeloma and other LP	Multiple myeloma *		Multiple myeloma and other LP*	
	n (%)	n (%)	n (%)	HR	95%CI	HR	95% CI
Fish							
≤ 2 portions p/w	149 (29.2)	7 (41.2)	10 (35.7)	1.00		1.00	
> 2 portions p/w	361 (70.8)	10 (58.8)	18 (64.3)	0.62	0.24–1.64	0.77	0.35–1.67
Fish oil							
less than weekly	152 (29.9)	6 (35.3)	9 (32.1)	1.00		1.00	
weekly or more	357 (70.1)	11 (64.7)	19 (64.9)	0.81	0.30–2.22	0.91	0.41–2.03
Salted fish							
less than once a month	371 (72.7)	13 (76.5)	21 (75.0)	1.00		1.00	
once a month or more	139 (27.3)	4 (23.5)	7 (25.0)	0.95	0.30–2.95	1.00	0.42–2.39
Meat							
2 times p/w or less	186 (36.4)	5 (29.4)	6 (21.4)	1.00		1.00	
3 times p/w or more	325 (63.6)	12 (70.6)	22 (78.6)	1.47	0.51–4.21	2.18	0.88–5.41
Smoked/salted meat							
less than once a month	341 (66.7)	14 (82.4)	20 (71.4)	1.00		1.00	
once a month or more	170 (33.3)	3 (17.6)	8 (28.6)	0.47	0.13–1.68	0.84	0.36–1.96
Milk and milk products							
less than daily	223 (43.7)	8 (47.1)	10 (35.7)	1.00		1.00	
daily	287 (56.3)	9 (52.9)	18 (64.3)	0.96	0.37–2.50	1.56	0.72–3.39
Fruits							
2 times p/w or less	151 (29.5)	9 (52.9)	13 (46.4)	1.00		1.00	
3 times p/w or more	360 (70.5)	8 (47.1)	15 (53.6)	0.34	0.13–0.89	0.45	0.21–0.96
Vegetables							
2 times p/w or less	270 (52.9)	11 (64.7)	16 (57.1)	1.00		1.00	
3 times p/w or more	240 (47.1)	6 (35.3)	12 (42.9)	0.54	0.20–1.49	0.78	0.36–1.67
Rye bread/flatbread							
2 times p/w or less	225 (44.4)	10 (58.8)	15 (53.6)	1.00		1.00	
3 times p/w or more	282 (55.6)	7 (41.2)	13 (46.4)	0.57	0.22–1.52	0.70	0.33–1.48
Sausage/liver							
never	111 (21.8)	3 (17.6)	6 (21.4)	1.00		1.00	
ever	398 (78.2)	14 (82.4)	22 (78.6)	1.40	0.40–4.88	1.07	0.43–2.64
Oatmeal/muesli							
2 times p/w or less	265 (51.9)	9 (52.9)	17 (60.7)	1.00		1.00	
3 times p/w or more	246 (48.1)	8 (47.1)	11 (39.3)	0.96	0.36–2.51	0.69	0.32–1.50
Potatoes							
less than daily	220 (43.1)	7 (41.2)	12 (42.9)	1.00		1.00	
daily	290 (56.9)	10 (58.8)	16 (57.1)	1.12	0.42–2.96	1.04	0.49–2.20
Whole wheat bread							
4 times p/w or less	130 (25.5)	4 (23.5)	4 (14.3)	1.00		1.00	
5 times p/w or more	380 (74.5)	13 (76.5)	24 (85.7)	1.02	0.33–3.15	1.93	0.66–5.56

*Models adjusted for age and sex.

Abbreviations: MGUS—Monoclonal gammopathy of undetermined significance, LC-MGUS–Light chain monoclonal gammopathy of undetermined significance, LP–lymphoproliferative, OR—Odds ratio, CI—confidence interval.

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high intake of fruit in our study was three times per week or more. This would normally be considered low intake, but due to the low frequency of fruit consumption the cut-off could not

be higher. The beneficial threshold for both risk of MGUS and progression to MM may therefore be low. Since access to fruit was limited in Iceland, especially during the adolescent period for this population and in rural areas, it is possible that fruit intake was an indicator of higher social status or overall healthier life-style, and therefore the higher intake individuals were at lower risk for multiple health outcomes, including MGUS and MM.

Interestingly, we did not find an association between intakes of fish and fish liver oil and MGUS or progression to MM. Although not significant, the point estimates in the progression analysis were in the same direction as previous studies on the association between fish intake and risk of MM have reported. A few case-control studies have reported an inverse association between fish intake and risk of MM [21, 24–26], including two that have found a dose-response relationship [21, 25]. Both of these studies report much lower fish intake than reported in our study. A suggested mechanism for the association is the cancer preventive effect of the polyunsaturated omega-3 essential fatty acid (n-3) [46]. Fish intake is a common indicator for social status [47]. However, for the Icelandic population, fish was widely available and the diet was characterized by a very high fish intake. Due to the high intake we do not have a non-exposed reference group in our fish intake analysis. Although our population has a uniquely high intake of fish, the most common types were lean fish, low in n-3. Nevertheless, the Icelandic population has high levels of the marine derived n-3 in both diet and plasma, even those with low intake of fish liver oil, a common supplement in Iceland, rich in n-3.[48] Therefore, we cannot rule out the option that a possible beneficial threshold of n-3 might already have been reached by our low intake fish and fish liver oil reference groups. Another reason for not finding an association between fish consumption and MM in our study could be the lack of a reference group in the study that did not consume fish.

We found that intake of whole wheat bread at least five time per week was inversely associated with MGUS when compared to lower intake. To date, only one study has analyzed the association between whole grain intake and risk of MM. A case-control study based on 120 MM cases below the age of 75 years reported that high intake of whole grain foods was inversely associated with MM risk in women (OR = 0.5, $p \leq 0.05$) but not in men [49]. Potential mechanisms that could mediate the effect of whole grains on MM risk have not been sufficiently explored. However, whole grains have been suggested to have positive effects on long-term insulin secretion [50] which could be of importance regarding MM since increased availability of IGF-1 can increase MM cell proliferation and prevent apoptosis [51].

We found inconsistency in LC-MGUS risk between our primary and sensitivity analyses when analyzing associations with fish, meat, and rye bread and flatbread intake and additionally when analyzing the association between rye bread and flatbread and potatoes and MGUS (MGUS and LC-combined) in our cross-classification analysis. The prevalence of LC-MGUS was 4.8% in our cohort, which is considerably higher than has been noted in previous studies [4, 5]. Although this is an elderly population the difference is substantial. It is therefore difficult to draw conclusions from the results.

The strength of our study is the well-established population-based cohort design with limited threat to both internal and external validity. Another major strength is the ability to study dietary intake throughout the lifespan, as studying early life exposures is challenging yet also important since many diseases originate early in life. Few studies have been able to provide data on adolescent or midlife diet combined with detailed ascertainment of later life health outcomes and we believe our study is unique in that aspect. Additionally, a major strength is the utilization of a validated food frequency questionnaire since majority of the questions had an acceptable correlation and the questionnaire was found suitable to rank individuals by their intake for most food groups when compared to a reference method [34, 35].

The mean age of our study population is 77 years and our cohort might represent a selection of participants that are healthier than the general population. Participants had to recall their dietary habits many decades back in time which could result in a misclassification of intake. Although, a previous study showed that food-related memory from childhood can be as accurate as from current diet, particularly for foods items eaten daily or rarely [52]. However, participants did not know their MGUS status at the time of questioning, and we therefore assume that the misclassification is non-differential. We did not have data on family history of hematologic cancers or information on total energy intake and were therefore unable to adjust for these factors. We do not know the true time of MGUS onset and therefore a misclassification of follow-up times could be present in our progression analysis, however, the prevalence of MGUS increases with age and by adjusting all our models for age we try to reduce the effect of this misclassification. Another limitation is lack of correlation to the reference method for midlife and late life intake of few food groups when the questionnaire was validated, possibly due to the inability of the food record used as reference method to adequately reflect individual intake of food items that are consumed less than four times a week [34, 35]. Validating early diet does pose great challenges and it is expected that such studies are unable to follow ideal procedures. Interpretation of our results are therefore limited by the results of the validation studies. Adolescent diet has not and cannot be validated, however, the data show similar frequency of intake according to residence in rural and coastal fishing areas, as documented in an Icelandic household study from 1939 [28, 53]. We cannot truly distinguish what could be smoldering multiple myeloma as we do not have bone marrow samples. Lastly, we cannot rule out the option that our findings are due to chance. Some of our results from the adolescent and midlife periods are limited by few number of MGUS or LC-MGUS cases in some categories, our progression analyses are additionally limited by few number of cases. Little is known about the relationship between diet and MGUS/LC-MGUS and progression to MM and this study can therefore be considered a hypothesis-generating study. Adjusting for multiple comparison is thought of as an insurance policy against mistakenly rejecting a null hypothesis, given that the null hypothesis is correct [54]. Due to the number of tests performed in the study the chances of rejecting a null hypothesis and obtaining positive results is high. However, the nature of our study is to seek potential risk factors for MGUS/LC-MGUS and progression to MM and we did not adjust for multiple testing in order not to increase the risk of missing out on possible risk factors.

To conclude, in this population-based screening study we found that high intake of fruit during the adolescent period and whole wheat bread during the midlife period may reduce the risk of MGUS later in life and that high fruit intake in late life may reduce the risk of progressing from MGUS/LC-MGUS to MM. Our findings suggest that food intake might alter the risk of developing MGUS and progressing to MM. Future studies should focus on clarifying the possible role of dietary habits in the pathogenesis of MGUS and MM.

Supporting information

S1 Table. Longitudinal effect of adolescent and midlife consumption of selected types of food on MGUS.
(DOCX)

S2 Table. Longitudinal effect of adolescent and midlife consumption of selected types of food on risk of MGUS using higher cutoff values for involved light chains in LC-MGUS cases.
(DOCX)

S1 File. Survey questionnaire.
(PDF)

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Author Contributions

Data curation: Gudny Eiriksdottir, Lenore J. Launer, Vilmundur Gudnason, Tamara B. Harris.

Formal analysis: Marianna Thordardottir.

Funding acquisition: Sigurdur Y. Kristinsson.

Investigation: Rene Costello, Debra Burton.

Methodology: Marianna Thordardottir, Ebba K. Lindqvist, Sigrun H. Lund, Laufey Steingrimsdottir, Johanna E. Torfadottir, Sigurdur Y. Kristinsson.

Project administration: Sigurdur Y. Kristinsson.

Resources: Rene Costello, Debra Burton.

Supervision: Johanna E. Torfadottir, Sigurdur Y. Kristinsson.

Writing – original draft: Marianna Thordardottir.

Writing – review & editing: Ebba K. Lindqvist, Sigrun H. Lund, Rene Costello, Laufey Steingrimsdottir, Neha Korde, Sham Mailankody, Gudny Eiriksdottir, Vilmundur Gudnason, Ola Landgren, Johanna E. Torfadottir, Sigurdur Y. Kristinsson.

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Paper III

Adolescent diet and risk of monoclonal gammopathy of undetermined significance

Marianna Thordardottir[1], Bryndis E Birgisdottir[2], Laufey Steingrimsdottir[2], Ebba K Lindqvist[3], Thor Aspelund[4], Rene Costello[5], Debra Burton[5], Neha Korde[6], Sham Mailankody[6], Gudny Eiriksdottir[7], Lenore J Launer[8], Tamara B Harris[8], Ola Landgren[6], Vilmundur Gudnason[7], *Sigurdur Y Kristinsson[1], and *Johanna E Torfadottir[4]

[1]Faculty of Medicine, University of Iceland, Reykjavik, Iceland.
[2]Unit for Nutrition Research, University of Iceland, Reykjavik, Iceland.
[3]Department of Medicine, Division of Hematology, Karolinska University Hospital and Karolinska Institutet, Stockholm, Sweden.
[4]The Centre of Public Health Sciences, University of Iceland, Reykjavik, Iceland.
[5]Multiple Myeloma Section, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA.
[6]Myeloma Service, Division of Hematologic Oncology, Memorial Sloan-Kettering Cancer Center, New York, NY, USA.
[7]Icelandic Heart Association, Kopavogur, Iceland.
[8]National Institute on Aging, National Institutes of Health, Bethesda, MD, USA.

* These authors contributed equally to this work

Corresponding author
Marianna Thordardottir
Faculty of Medicine
University of Iceland
mthordar@hi.is

Abstract

Background: Few studies on the association between diet and multiple myeloma have been conducted and the results have been inconclusive. We recently found that diet is associated with the premalignant state, monoclonal gammopathy of undetermined significance (MGUS). The aim of this study was to examine whether dietary patterns in adolescence are associated with MGUS.

Methods: This study was based on participants from the AGES Reykjavik Study (N = 5,764). The participants were born during the years of 1907-1935 and during the years of 2002-2006 they gave retrospective information on frequency of intake of common food items in adolescence (14 - 19 years). Principal component analysis was used to extract dietary patterns and the participants were ranked according to their adherence to each pattern extracted. All participants were screened for MGUS at study entry.

Results: A total of 300 (5.2%) MGUS cases and 275 (4.8%) light-chain MGUS cases were identified. The two dietary patterns with the highest variance was firstly a pattern including salted/smoked meat and fish, blood or liver sausage, rye bread, and milk (Traditional early 20th century diet) and secondly a pattern including fruit and vegetables (Healthy diet). High adherence to the Traditional early 20th century diet was inversely associated with MGUS and LC-MGUS combined (odds ratio (OR) = 0.65, 95% confidence interval (CI) 0.51-0.84) and MGUS (OR = 0.65, 95% CI 0.46-0.91).

Conclusion: Our findings suggest that high adherence to a food pattern including salted/smoked meat and fish, blood or liver sausage, rye bread, and milk in adolescence is associated with decreased risk of MGUS later in life. The findings indicate that food and nutrition intake during the vulnerable period of adolescence might play a role in preventing the development of conditions such as MGUS.

Introduction

Monoclonal gammopathy of undetermined significance (MGUS) is a precursor condition preceding multiple myeloma.[1, 2] MGUS is an asymptomatic condition characterized by the presence of an M-protein in serum or by an abnormal free light chains (FLC) ratio, increased concentration of the involved FLC, and lack of intact immunoglobulin (light-chain MGUS), without evidence of multiple myeloma or other lymphoproliferative disorders.[3-5] The prevalence of MGUS increases with age, it is approximately 5% in those older than 70 years, and is higher in men than in women.[6] The average risk of progression from MGUS to multiple myeloma is estimated to be 1% per year.[1, 7]

The etiology of MGUS and light-chain MGUS (LC-MGUS) is to a large extent unknown. Studies have found that it is more prevalent among the black race,[8, 9] in those with a family history of MGUS and related diseases,[10] and for those with prior personal and family history of immune-related conditions.[11, 12] The impact of the environment on MGUS has in recent times gained attention and a study from 2015 found a positive association between exposure to Agent Orange, herbicide and defoliant chemical, and MGUS.[13] Obesity has consistently been identified as a risk factor for multiple myeloma,[14, 15] and the International Agency for Research on Cancer recently concluded that there is now sufficient evidence for the association between increased body weight and 13 types of cancers, including multiple myeloma.[16] To date, three studies on the association between obesity and MGUS have been conducted. One found an association between obesity (BMI >30 kg/m^2) and MGUS in women,[17] while other two did not find any association.[9, 18] However, we recently found that high BMI during the midlife period (age 40 - 50 years) is associated with increased risk of progressing from MGUS to multiple myeloma later in life.[18] Epidemiological evidence on the association between diet and multiple myeloma is scarce and the results are inconclusive. The strongest evidence exists for fish intake, where inverse association has been reported in a few case-control studies.[19-22] In a recently published study we found that intake of fruit at least three times per week during the adolescent years (age 14 - 19 years) and whole wheat bread at least five times per week during the midlife period was inversely associated with MGUS when compared to lower intake, and additionally that same amount of fruit intake after MGUS onset was inversely associated with progression to multiple

myeloma and related diseases.[23] This suggests that dietary habits early in life might play a role in the pathogenesis of multiple myeloma and related diseases. Studies have shown that the adolescent period is a sensitive period with regard to nutrition and cancer risk.[24-28] That is consistent with migrant studies showing that it takes only one generation to gain the cancer risk of the host country, implying that early life environmental factors, such as diet, might play a role in cancer risk later in life.[29-32] Additionally, MGUS has been detected in individuals as young as 10 - 19 years old, [33] emphasizing the importance of studying the association between early life exposures and MGUS.

Most studies to date use foods, food groups, or nutrient intakes as exposures when analyzing the relationship between diet and diseases of interest. However, studying dietary patterns offers a broader view of food consumption and possible effects of the whole diet on diseases since many nutrients and other substances in foods might act together in the development of different diseases. To better understand the complex association between diet and risk of MGUS and LC-MGUS we aimed to explore whether adherence to empirically derived dietary patterns from the adolescent period (14 - 19 years) was associated with risk of these precursor diseases later in life using data from a population-based cohort of elderly individuals.

Materials and Methods

Study population

This study is based on participants from the population-based Age, Gene/Environment Susceptibility (AGES) Reykjavik Study (N = 5,764; born in 1907 - 1935), conducted during the years of 2002-2006 by the Icelandic Heart Association. Detailed description of the study and collection of data has been previously published.[34] The AGES Reykjavik Study is a follow-up of the Reykjavik Study. All men and women residing in the Reykjavik area in 1967 were invited to participate during the years of 1967-1996. In March 2002, when the AGES Reykjavik Study was initiated, 11,549 cohort members from the Reykjavik Study were still alive, of which 8,030 were randomly chosen and invited to participate. A total of 5,764 (71.8%) individuals consented to participate by 2006, when the study ended. Extensive data

were collected in the study during clinical examination, including data on dietary intake during adolescence, midlife, and current late life intake.[34]

Participants signed an informed consent form and the study was approved by the Icelandic National Bioethics Committee (VSN-00-063-V35), the Icelandic Data Protection Authority, and the Institutional Review Board of the National Institute on Aging in the USA. A total of 39 (0.7%) subjects were excluded from analysis in this study, 21 due to previous lymphoproliferative disorders, 16 due to missing blood sample, one due to missing consent form, and one subject had high M-protein concentration at baseline and therefore fulfilled criteria for smoldering multiple myeloma.

Dietary assessment

At study entry the participants provided retrospective information on dietary habits during adolescence (14 - 19 years) and midlife (40 - 50 years), as well as information on current dietary habits using a food frequency questionnaire. The questionnaire included questions regarding frequency of intake of common foods and food groups from each period, i.e. fish (including salted and smoked fish), fish oil, meat (including salted and smoked meat), milk and milk products, fruit, vegetables, rye- and flatbread, blood or liver sausage, oatmeal and muesli, and potatoes.

The questionnaire has been validated for the midlife and late life periods, and was found suitable to rank individuals according to their intake for most food groups included in the questionnaire.[35, 36] For the current study information on dietary habits during adolescence is used. As information on adolescent diet was collected retrospectively several decades back in time, this period of the food frequency questionnaire has not been validated. However, the participants gave information on place of residence when growing up and their dietary data show similar frequency of intake of different food items according to residence in rural and coastal fishing areas, as documented in an Icelandic household dietary study from 1939.[24, 37]

Extraction of dietary patterns

Principal component analysis (PCA) was used to extract dietary patterns. This method is data driven and forms new linear factors (dietary patterns) by reducing

data dimension and grouping correlated variables (food intake).[38] The components extracted by this method reflect combinations of food groups consumed by the participants. The correlation coefficients defining the components are called factor loadings and represent the correlation between each input variable (foods or food groups) and the extracted components. A positive factor loading indicates that the corresponding foods or food groups are positively correlated with the dietary pattern, whereas negative factor loadings indicate an inverse correlation. The number of factors to retain was based on interpretability of the variance, eigenvalues (>1.5), and the scree plot (Supplementary figure 1). Food groups with factor loadings higher than 0.30 or lower than -0.30 were considered important for the interpretability of the dietary patterns.[39] For each pattern extracted a new variable was created, ranking participants on their adherence to that particular pattern. A low score indicates low adherence to the pattern and a high score indicates high adherence. The higher the adherence, the more closely the participant´s diet conforms to that pattern.

Identification of MGUS cases

During the years of 2013-2014 serum samples from all participants, collected at AGES Reykjavik Study entry, was shipped from Iceland to the Multiple Myeloma Research laboratory at the National Cancer Institute in the United States, where protein and free light chain assays were performed. MGUS cases were defined as having M-protein bands on serum protein electrophoresis (SPEP) and an M-protein concentration below 30 g/L (heavy chain MGUS).[5] Samples with an equivocal or definite M-protein present on SPEP were then subjected to serum protein immunofixation for conformation and typing of the M-protein.[6] The criteria for LC-MGUS included no M-protein band visible on SPEP, an increased or decreased FLC ratio (<0.26 or >1.65) in combination with an increased concentration of the involved light chain (kappa >19.4 mg/L, lambda >26.3 mg/L).[40] All testing and interpretations were done by individuals blinded to all demographics and other details pertaining to the samples being tested.

Ascertainment of covariance

Information on potential confounders, such as age (continuous), gender, lifetime (20-65 years old) physical activity (never/rarely/occasionally, moderate/high), education

(primary/secondary, college, university) collected at study entry to the AGES Reykjavik Study was retrieved. Information on midlife body mass index (continuous) and early life residency (capital area, sea side, country side, combination of sea and country side) was retrieved from the Reykjavik Study. Classification of early life residency has been described in an earlier study.[24]

Statistical analysis

To test the sampling adequacy and the suitability of our data for factor analysis Bartlett's test of Sphericity ($p<0.0001$) and Kaiser-Mayer-Olkin test (0.71) were performed, denoting adequate sampling size and correlation of the variables. We used logistic regression to estimate odds ratios (OR) and 95% confidence intervals (CI) for MGUS and LC-MGUS according to adherence to the extracted dietary patterns. Each dietary pattern was divided into tertiles, and the lowest third was used as a reference category. Tests for linear trend were calculated by adding the tertiles of the factor score as an ordered variable into the multivariate models (p trend). We considered potential confounders and adjusted all our models for age and sex. We furthermore adjusted for BMI, physical activity, education, and residency in the second model. All analyses were performed in R version 3.4.0.[41]

Sensitivity analysis

The prevalence of LC-MGUS was unusually high in our cohort (4.8%) compared with other published studies (0.7-0.8%),[3, 4] mostly due to a high prevalence of kappa cases (96%). As previously described [18, 23, 42] the distribution of log transformed kappa and lambda concentration values resembled the normal distribution. The definition of LC-MGUS was therefore modified by using the 97.5th percentile as cut-off for normal kappa and lambda values (approx. 40.0 mg/L for both light chains), but the same pathological FLC ratio as previously described (< 0.26 and > 1.65). The modified definition resulted in 52 LC-MGUS cases (0.9%), whereof 41 (79%) were kappa and 11 were lambda. Using the modified definition, a sensitivity analysis on the association between adolescent dietary patterns and total MGUS, MGUS, and LC-MGUS was performed, including the same covariates as previously mentioned.

Results

Of the 5,725 available for analysis, 3,306 were female (57.7%), and the mean age at study baseline was 77 years (Table 1). A total of 575 (10.0%) MGUS cases were identified, of which 300 (5.2%) were heavy chain MGUS and 275 (4.8%) LC-MGUS.

Dietary patterns

Four components (dietary patterns) had an eigenvalue greater than one. However, there was a clear break in the scree plot (Supplementary figure 1) after the second component and therefore two patterns with eigenvalues >1.5 were retained. Table 2 shows the factor loading coefficients between the food groups and the extracted patterns and the cumulative variance explained by the patterns. The first pattern had an eigenvalue of 2.5 and was characterized by a high consumption of salted or smoked meat, salted or smoked fish, blood- or liver sausage, rye bread, milk and milk products, oatmeal and potatoes. This pattern was labelled "Traditional early 20th century diet." The second pattern had an eigenvalue of 1.8 and was characterized by a high consumption of fruit, vegetables, and fish on bread or in salad. This pattern was labelled "Healthy diet."

The Traditional early 20th century diet

In a fully adjusted model (Table 3) a statistically significant inverse association was found for both the mid and the highest tertile of adherence to the Traditional early 20th century diet and total MGUS (heavy chain MGUS and LC-MGUS combined) (OR = 0.75, 95% CI 0.59-0.96 and OR = 0.65, 95% CI 0.51-0.84, respectively, p for trend = 0.001) when compared to the lowest tertile of adherence. When analyzing heavy chain MGUS and LC-MGUS separately a statistically significant inverse association was found between the highest tertile of adherence and heavy chain MGUS (OR = 0.65, 95% CI 0.46-0.91, p for trend 0.013), and also for both the mid and the highest adherence and LC-MGUS (OR = 0.68, 95% CI 0.48-0.96 and OR = 0.66, 95% CI 0.46-0.94, respectively, p for trend 0.021) when compared to the lowest tertile of adherence.

The Healthy diet

In a fully adjusted model (Table 4) a statistically significant inverse association between the highest tertile of adherence to a Healthy diet and LC-MGUS was found (OR = 0.62, 95% CI 0.43-0.90, p for trend = 0.012) when compared to the lowest tertile. No association was found for total MGUS or heavy chain MGUS (Table 4).

Sensitivity analysis

With the modified LC-MGUS definition, the prevalence of LC-MGUS dropped from 4.8% (n = 275) to 0.9% (n = 52). In a fully adjusted model (Supplementary table 1) we found that the highest adherence to the Traditional early 20th century diet was, as before, inversely associated with total MGUS and MGUS (OR = 0.62, 95% CI 0.45-0.85 and OR = 0.66, 95% CI 0.46-0.93, respectively). We also found a dose response for both total MGUS and MGUS as we did in our primary analysis (p for trend 0.003 and 0.016, respectively). We additionally found that the Traditional early 20th century diet was associated with LC-MGUS, both the mid and the highest tertile (OR = 0.37, 95% CI 0.16-0.87 and OR = 0.44, 95% CI 0.20-0.98, p for trend 0.044). We found no association between the Healthy diet and total MGUS, heavy chain MGUS, or LC-MGUS (supplementary table 2).

Discussion

In this population-based study, including data from 5,725 individuals, we found that high adolescent exposure to a Traditional early 20th century dietary pattern, characterized by high intake of salted or smoked meat and fish, blood or liver sausage, rye bread, milk and milk products, oatmeal, and potatoes, was associated with a significant decreased risk of MGUS and LC-MGUS. Our findings suggest that early life dietary intake influences the etiology of MGUS and adds to the evolving literature on lifestyle related factors in the pathogenesis of MGUS.

We found that high adherence to the Traditional early 20th century diet was associated with decreased risk of MGUS. These results were surprising as some of the food components found in this pattern have been positively associated with various types of cancer, such as red and processed meat.[43] In our previous study,

we analyzed the individual food components within the same cohort and found none of the foods included in this pattern to be associated with MGUS.[23] There we found that high adolescent intake of fruit and high midlife intake of whole grain bread was associated with reduced risk of MGUS and that high late life intake of fruit in individuals with MGUS or LC-MGUS reduced the risk of progressing to multiple myeloma and other lymphoproliferative diseases. The association between this pattern and risk of MGUS was therefore stronger than for individual food components in the pattern, suggesting that the benefit of a combined dietary factors is stronger than for isolated food items. Although it is a strength to analyze the diet as a whole, one of the weaknesses is of this method is that the possible association might be due to only one or two food components in the pattern, transferring the effect to all the components. Also, there might be a component found in the pattern that is associated with the outcome but is missed when the diet as a whole is analyzed, as we see with fruit in the Healthy diet. The mechanisms for our findings are unknown, but is maybe further evidence for the effect of environmental factors in the pathogenesis of MGUS. Due to the relative isolation of many residential regions in Iceland during the first half of the 20th century, there was a considerable variation in diet across the country. The diet was however, largely limited to locally produced food available on site and we observe in our data that people with the highest adherence to the Traditional early 20th century diet are participants growing up in the rural areas, such as farmers. However, adjusting for residency did not affect our results. We therefore speculate whether the residency from that period is perhaps a proxy for something else that could affect the risk, for example physical activity. The association between physical activity and MGUS has to our knowledge not been investigated before and results on the association between physical activity and multiple myeloma are scarce and inconsistent.[44-47] A systematic review and meta-analysis from 2014 reported a marginally significant association (risk ratio = 0.86, 95% CI 0.68-1.09) between leisure time physical activity and multiple myeloma,[45] while a recent study on three large cohorts found no association between cumulative average physical activity and walking and risk of MM.[46] We do have information on physical activity, and adjusted for it, however, they are limited since we do not have an estimate of physical activity at exact time of exposure, only lifetime activity from 20 - 65 years old. Although only 30% of farmers report high lifetime physical activity,

we do know that farming required a lot of physical strength that perhaps results in less leisure time physical activity.

We found that high adherence to the Traditional early 20th century diet and the Healthy diet was inversely associated with LC-MGUS. However, these results were not fully supported by our sensitivity analysis where we did no find the Healthy diet to be associated with LC-MGUS. The prevalence of LC-MGUS was 4.8% in our cohort, which is considerably higher than has been noted in previous studies.[3, 4] Although this is an aged population the difference is substantial. The number of cases dropped from 275 to 52 with the modified definition of LC-MGUS and the discrepancy between the primary and sensitivity analysis with regards to the Healthy diet could be due to a loss of power and should therefore be interpreted with caution.

The major strength of our study is the population-based cohort design and extensive information on covariates, making adjustment for potential confounders available. Another strength is the ability to study early life dietary exposure, although studying early life exposures is challenging it is also very important since many diseases originate early in life. Few studies have been able to provide data on early life diet, combined with detailed ascertainment of later life health outcomes and we believe our study is unique in that aspect. Additionally, using empirically derived eating patterns in addition to analyzing each food component separately gives a better understanding of the possible association between diet and MGUS. This method accounts for complex interactions among nutrients and foods and has the advantage of describing the ways that foods are combined in actual diets.

Some limitations need to be kept in mind when interpreting the results. The dietary data are based on retrospective data collection which could result in a recall bias and therefore a misclassification of exposure might be present. However, we consider the potential misclassification to be non-differential since participants did not know their MGUS status at time of questioning. Additionally, a previous study showed that food-related memory from childhood can be as accurate as from current diet, particularly for foods items eaten daily or rarely.[48] The food frequency questionnaire has not been validated for the adolescent period and cannot be done due to the retrospective nature of the assessment. However, our data show similar frequency of intake of the most commonly consumed foods from that time period as documented in an Icelandic household study from 1938.[24, 37] Additionally, a selection bias might be present. The mean age of our population is 77 years and therefore the cohort might

represent a selection of the population that is healthier than the general population. A limitation of the method used is that the possible association might be due to only one or two food components in the pattern, but the it is transferred to all the components, and conversely, the effect of a single nutrient or food component might be missed when analyzing the diet as a whole. We therefore use it as a complimentary approach to our previous study on the association between single food groups and MGUS. Lastly, we do not have bone marrow samples from our participants and therefore cannot truly distinguish MGUS from what could be considered as smoldering multiple myeloma.

To conclude, in this population-based study we found that high adherence to a dietary pattern characterized by high intake of salted or smoked meat, salted or smoked fish, blood or liver sausage, rye bread, milk and milk products, oatmeal, and potatoes was inversely associated with MGUS. These findings indicate that food intake during a vulnerable period with regard to nutrition, might alter the risk of developing MGUS. Future studies should aim to clarify the possible mechanism of diet in the pathogenesis of MGUS.

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Table 1 Characteristics of study participants

	Total	Traditional early 20th century diet			
	n = 5,725	Lowest tertile n = 1,701	Middle tertile n = 1,754	Highest tertile n = 1,702	p value
Age, mean (SD)	77.0 (5.9)	75.6 (5.3)	76.3 (5.4)	77.6 (5.8)	<0.001
Sex, n (%)					
Females	3306 (57.7)	1060 (62.3)	677 (55.7)	902 (53.0)	
Males	2419 (42.3)	641 (37.7)	777 (44.3)	800 (47.0)	<0.001
BMI in midlife, mean (SD)	25.2 (3.59)	25.2 (3.6)	25.2 (3.5)	25.3 (3.6)	0.643
Physical activity, n (%)					
Never/rarely/occasionally	3362 (67.7)	1100 (69.1)	1106 (66.8)	1068 (66.8)	
Moderate/high	1606 (32.3)	493 (30.9)	550 (33.2)	532 (33.3)	0.280
Healthy diet, n (%)					
Tertile 1	1701 (33.0)	596 (35.1)	501 (28.6)	603 (35.4)	
Tertile 2	1754 (34.0)	579 (34.1)	600 (34.2)	575 (33.8)	
Tertile 3	1702 (33.0)	525 (30.9)	653 (37.2)	524 (30.8)	<0.001
Residency					
Reykjavik area	2121 (37.8)	784 (47.2)	748 (43.4)	392 (23.6)	
Seaside village	1861 (33.0)	589 (35.4)	611 (35.4)	488 (29.4)	
Rural area	1432 (25.5)	246 (14.8)	310 (18.0)	707 (42.6)	
Combination of rural and seaside	191 (3.4)	43 (2.6)	56 (3.2)	71 (4.3)	<0.001
Education					
Primary and secondary	3905 (73.6)	1212 (71.3)	1273 (72.8)	1290 (76.1)	
College	809 (15.2)	282 (16.6)	274 (15.7)	230 (13.6)	
University	594 (11.2)	205 (12.1)	201 (11.5)	175 (10.3)	0.033

Table 2 Factor loadings coefficients between the food groups and the extracted dietary patterns

Dietary pattern	Food	Factor loading coefficient	Cumulative variance explained (%)*
Traditional early 20th century diet	Salted/smoked meat	0.78	19
	Salted/smoked fish	0.73	
	Blood sausage/liver sausage	0.59	
	Rye bread	0.47	
	Milk & milk products	0.42	
	Oatmeal	0.35	
	Potatoes	0.30	
Healthy diet	Fruit	0.77	33
	Vegetables	0.76	
	Fish on bread or in salad	0.64	

Factor loadings are correlation coefficients between input variables (foods) and extracted factor. Food groups are sorted by size of loading coefficients. For simplicity food groups with factor loadings between 0.30 and -0.30 are not listed.
*Percentage of variance in total food intake explained by patterns.

Table 3 Association between adherence to the Traditional early 20th century diet and MGUS and LC-MGUS

Traditional early 20th century diet	Cases (n)	[a]Model 1 OR (95% CI)	p trend	[b]Model 2 OR (95% CI)	p trend
All MGUS (n= 502)					
Tertile 1	185	1.00 (ref.)		1.00 (ref.)	
Tertile 2	163	0.77 (0.62-0.97)		0.75 (0.59-0.96)	
Tertile 3	154	0.67 (0.53-0.85)	<0.001	0.65 (0.51-0.84)	0.001
MGUS (n= 264)					
Tertile 1	95	1.00 (ref.)		1.00 (ref.)	
Tertile 2	92	0.86 (0.64-1.16)		0.82 (0.60-1.13)	
Tertile 3	77	0.68 (0.50-0.93)	0.016	0.65 (0.46-0.91)	0.013
LC-MGUS (n= 238)					
Tertile 1	90	1.00 (ref.)		1.00 (ref.)	
Tertile 2	71	0.68 (0.49-0.94)		0.68 (0.48-0.96)	
Tertile 3	77	0.67 (0.49-0.92)	0.014	0.66 (0.46-0.94)	0.021

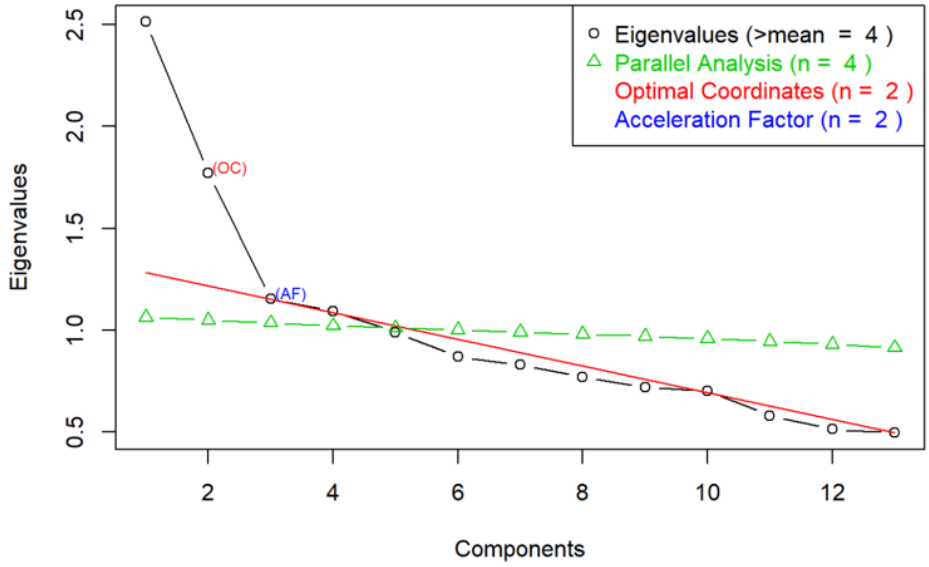
[a]Adjusted for age at study baseline and sex
[b]Adjusted for age at study baseline, sex, BMI, physical activity, education, and residency

Table 4 Association between adherence to the Healthy diet and MGUS and LC-MGUS

Healthy diet	Cases (n)	[a]Model 1 OR (95% CI)	p trend	[b]Model 2 OR (95% CI)	p trend
All MGUS (n= 502)					
Tertile 1	181	1.00 (ref.)		1.00 (ref.)	
Tertile 2	173	1.01 (0.81-1.26)		0.98 (0.77-1.25)	
Tertile 3	148	0.92 (0.73-1.16)	0.463	0.88 (0,68-1.13)	0.320
MGUS (n= 264)					
Tertile 1	82	1.00 (ref.)		1.00 (ref.)	
Tertile 2	92	1.16 (0.85-1.59)		1.10 (0.79-1.54)	
Tertile 3	90	1.20 (0.88-1.64)	0.245	1.18 (0.84-1.67)	0.335
LC-MGUS (n= 238)					
Tertile 1	99	1.00 (ref.)		1.00 (ref.)	
Tertile 2	81	0.88 (0.65-1.19)		0.88 (0.63-1.22)	
Tertile 3	58	0.67 (0.48-0.94)	0.021	0.62 (0.43-0.90)	0.012

[a]Adjusted for age at study baseline and sex
[b]Adjusted for age at study baseline, sex, BMI, physical activity, education, and residency

Supplementary figure 1: Scree plot of adolescent dietary patterns



Supplementary table 1: Association between adherence to the Traditional early 20th century diet and MGUS using the modified definition of LC-MGUS

Traditional early 20th century diet	Cases (n)	[a]Model 1 OR (95% CI)	p trend	[b]Model 2 OR (95% CI)	p trend
All MGUS (n= 305)					
Tertile 1	114	1.00 (ref.)		1.00 (ref.)	
Tertile 2	100	0.78 (0.59-1.03)		0.75 (0.56-1.01)	
Tertile 3	91	0.66 (0.49-0.88)	0.005	0.62 (0.45-0.85)	0.003
MGUS (n= 264)					
Tertile 1	95	1.00 (ref.)		1.00 (ref.)	
Tertile 2	92	0.87 (0.65-1.17)		0.83 (0.61-1.14)	
Tertile 3	77	0.69 (0.50-0.94)	0.020	0.66 (0.46-0.93)	0.016
LC-MGUS (n= 41)					
Tertile 1	19	1.00 (ref.)		1.00 (ref.)	
Tertile 2	8	0.35 (0.15-0.80)		0.37 (0.16-0.87)	
Tertile 3	14	0.52 (0.25-1.05)	0.068	0.44 (0.20-0.98)	0.044

[a]Adjusted for age at study baseline and sex
[b]Adjusted for age at study baseline, sex, BMI, physical activity, education, and residency

Supplementary table 2: Association between adherence to the Healthy diet and MGUS using the modified definition of LC-MGUS

Healthy diet	Cases (n)	[a]Model 1 OR (95% CI)	p trend	[b]Model 2 OR (95% CI)	p trend
All MGUS (n= 502)					
Tertile 1	99	1.00 (ref.)		1.00 (ref.)	
Tertile 2	109	1.17 (0.88-1.56)		1.15 (0.85-1.56)	
Tertile 3	97	1.12 (0.83-1.50)	0.464	1.14 (0.83-1.58)	0.406
MGUS (n= 264)					
Tertile 1	82	1.00 (ref.)		1.00 (ref.)	
Tertile 2	92	1.18 (0.86-1.60)		1.14 (0.82-1.59)	
Tertile 3	90	1.22 (0.90-1.67)	0.203	1.22 (0.87-1.71)	0.253
LC-MGUS (n= 238)					
Tertile 1	17	1.00 (ref.)		1.00 (ref.)	
Tertile 2	17	1.17 (0.59-2.32)		1.25 (0.61-2.57)	
Tertile 3	7	0.53 (0.22-1.30)	0.166	0.68 (0.27-1.73)	0.421

[a]Adjusted for age at study baseline and sex
[b]Adjusted for age at study baseline, sex, BMI, physical activity, education, and residency