A PROSPECTIVE ANALYSIS OF THE RELATIONSHIP BETWEEN FISH INTAKE AND CENTRAL ADIPOSITY

by

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I dedicate this manuscript to my husband Justin and our son Jackson. They have given me a level of love, patience, and encouragement during this time that I will never be able to repay, but will always be grateful for - I love you guys!

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ABSTRACT

Accumulation of visceral adipose tissue, or central adiposity, increases prevalence of many chronic diseases and mortality, this relationship is independent of total of body fat. Central adiposity is commonly defined as having a waist circumference of > 102 cm in men and > 88 cm in women. Increased fish intake may have a beneficial effect on central adiposity. This prospective study examined the longitudinal relationship between fish intake at baseline and central adiposity over time.

Data were drawn from the Osteoarthritis Initiative (OAI). The sample included 3,033 participants (56.8% women, 83.3% white, *M* age = 61.4 years). Fish intake (cups/week) was collected at baseline with a food frequency questionnaire and examined as non-fried fish and fried fish separately. Central adiposity was measured as waist circumference (cm) and assessed at baseline, 24 months and 48 months.

Statistical analyses were carried out using generalized estimating equations (GEE). Associations between fish intake and waist circumference across time were adjusted for age, race, sex, energy intake, smoking status, presence of knee osteoarthritis, clinic assignment, level of physical activity, level of depression, and time between visits.

Non-fried fish intake was negatively associated with waist circumference across time (B = -0.68, p < .001) and fried fish intake was positively associated with waist circumference across time (B = 1.69, p = .003). Additional positive associations with waist circumference were found for age, energy intake, level of depression, and time between visits. A negative association with waist circumference was found for level of physical activity. Smaller waist circumferences were observed in women compared to men and in Hispanic participants compared to white participants. Larger waist circumferences were seen in black participants compared to white participants and in participants who were symptomatic for knee osteoarthritis compared to those without symptomatic knee osteoarthritis. Smoking was not found to be a predictor of waist circumference across time.

These results suggest that dietary recommendations for fish intake should make a distinction between increasing non-fried fish in the diet and avoiding fried fish. Results also suggest that symptomatic knee osteoarthritis has a strong relationship with waist circumference. Health professionals working with osteoarthritis patients should assess and monitor for central adiposity.

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n-3 PUFA …….…......	Omega-3 polyunsaturated fatty acid
AHA …………………...	American Heart Association
AIC …………………….	Akaike's information criterion
ALA ……………………	*alpha*-linolenic acid
AND …………………...	Academy of Nutrition and Dietetics
ATP III ………………...	Adult Treatment Panel III
BMI …………….……...	Body mass index
CES-D ………………...	Center for Epidemiological Studies Depression Scale
CT ………….………….	Computed tomography
DEXA ………………….	Dual-energy X-ray absorptiometry
DHA …………………...	Docosahexaenoic acid
EPA …………............	Eicosapentaenoic acid
EPIC …………………..	European Prospective Investigation in Cancer and Nutrition
FFQ ……………………	Food frequency questionnaire
GEE …………………...	Generalized estimating equations
GLM …………………...	Generalized linear models
LC n-3 PUFA …………	Long-chain omega-3 polyunsaturated fatty acid
MRI ……………………	Magnetic resonance imaging
MUFA …………………	Monounsaturated fatty acid

NCEP ……….……….	National Cholesterol Education Program
NHANES ……………...	National Health and Nutrition Examination Survey
NHLBI …………………	National Heart, Lung and Blood Institute
NIDDK ……..................	National Institute of Diabetes and Digestive and Kidney Diseases
NIH …………………….	National Institutes of Health
OAI …………………….	Osteoarthritis Initiative
PASE ………………….	Physical Activity Scale for the Elderly
PUFA ………………….	Polyunsaturated fatty acid
QICC ……….………..	Corrected Quasi Likelihood under Independence Model Criterion
QL ……………………..	Quasi-likelihood
SAT ……………………	Subcutaneous adipose tissue
SFA ……………………	Saturated fatty acid
SPSS ………………….	Statistical Package for Social Science
VAT ……………………	Visceral adipose tissue

CHAPTER ONE: INTRODUCTION

Obesity is linked to increased prevalence of heart disease (Patel, Winkel, Ali, Narayan, & Mehta, 2015), type 2 diabetes (Wang, Rimm, Stampfer, Willett, & Hu, 2005), and cancer (Bhaskaran et al., 2014), as well as increased mortality relative to those of normal weight (Flegal, Kit, Orpana, & Graubard, 2013; Masters et al., 2013). There is a lack of consistency in the definition of obesity, but most commonly the term refers to excess body weight for a given height and is measured in terms of body mass index (BMI) (Pi-Sunyer et al., 1998). BMI is easy to use in a clinical or community-based setting and is strongly correlated to total body fat and cardiovascular risk factors (Barreira et al., 2012), but BMI is a flawed measure because it does not provide any indictor of fat distribution (Kahn & Bullard, 2016).

Central adiposity (also called central or visceral obesity) is the presence of excess adipose tissue accumulated in the abdominal cavity and around the middle section of the body (Shuster, Patlas, Pinthus, & Mourtzakis, 2012). This visceral adipose tissue (VAT) is believed to be more metabolically active than fat that is more predominately distributed in the hips and thighs and as a result has a stronger influence on blood glucose control and other hormonal actions in the body and poses a greater health risk than the total amount of body fat (Bjorntorp, 1991; Fox et al., 2007; Katzmarzyk, Heymsfield, & Bouchard, 2013). While BMI and most other anthropometric measurements have been found to be highly correlated with total body fat, waist circumference has been determined to be the best practical measure to estimate the amount of VAT in an individual to access level of central adiposity (Barreira et al., 2012). The presence of central adiposity is usually indicated by a waist circumference of > 88 cm in women and > 102 cm in men. An elevated waist circumference is an independent risk factor for hypertension, diabetes, poor lipid profile and the metabolic syndrome (Barreira et al., 2012; Grundy et al., 2005) and is associated with higher mortality risks (Behrens et al., 2013; van der A, Nooyens, van Duijnhoven, Verschuren, & Boer, 2014). Waist circumference is a better predictor of mortality from specific causes than BMI (Leitzmann et al., 2011).

Central adiposity is a prevalent condition in the United States. Since 1988, prevalence of central adiposity has increased in both women and men, 0.37% per year and 0.27% per year respectively (Ladabaum, Mannalithara, Myer, & Singh, 2014). The largest increases were seen in young women and in overweight adults. Non-Hispanic black women and non-Hispanic white men were found to have the largest waist circumference compared to other race-gender groups (Fryar, Gu, & Ogden, 2012; Ladabaum et al., 2014). An examination of the 2007 – 2010 NHANES data found an average waist circumference of 95.2 cm in women over the age of 20 years and 100.9 cm in men over the age of 20 years (Fryar et al., 2012). Waist circumference increased with age until the age of 70 years in women and until the age of 80 years in men.

One strategy recommended to prevent and reduce incidence of obesity and central adiposity is to regularly engage in healthy behaviors, including enjoying a nutritious, energy-balanced diet (Department of Health and Human Services & Agriculture, 2015). While there is a clear link between the amount of energy a person consumes and body weight, the effects of individual components of the diet are not as well understood and the results of dietary interventions have had mixed (Katan & Ludwig, 2010).

Dietary fish intake became a topic of interest in health research after populations with higher than average fish intake were observed to have a low prevalence of cardiovascular disease (Bang, Dyerberg, & Sinclair, 1980; Middaugh, 1990). The marine-derived long chain omega-3 polyunsaturated fatty acids (LC n-3 PUFAs) found almost exclusively in fish and shellfish are essential for human health (Vannice & Rasmussen, 2014). There is evidence that higher fish intake is protective against cardiovascular disease risk factors, including hypertriglyceridemia (Marklund et al., 2015) and type 2 diabetes (P. S. Patel et al., 2009), metabolic syndrome (Zaribaf et al., 2014), and certain cancers (Turunen, Suominen, Kiviranta, Verkasalo, & Pukkala, 2014). A large study examining 36 countries found an inverse association between fish intake and all-cause mortality, ischemic heart disease and stroke (Zhang, Sasaki, Amano, & Kesteloot, 1999).

Predominantly based on the strength of the evidence of a beneficial relationship with heart disease, the general recommendation for healthy US adults is to consume fish at least twice per week, with a total intake of approximately 8 ounces per week (Department of Health and Human Services &

Agriculture, 2015). Evidence suggests that the average American does not eat the recommended amount of fish each week (Papanikolaou, Brooks, Reider, & Fulgoni, 2014).

There is evidence that increased fish intake may be beneficial for weight loss and decreased waist circumference. In 2014, the first meta-analysis of randomized controlled trails to examine the effects of increased fish and fish oil intake on anthropometric measures of body composition found significant effects compared to control group for waist circumference (-0.81 cm), body weight (-0.59 kg), BMI (-0.24 kg/m^2), and percent body fat (-0.49%) (Bender et al., 2014).

Purpose of the Study

The purpose of the current prospective study is to investigate the longitudinal relationships between baseline fish intake and repeated measurements of waist circumference in a sample of 3,033 men and women. The repeated measurements of waist circumference at baseline, 24 months follow-up and 48 months follow-up will allow for investigation of associations between fish intake measured at baseline and change in waist circumference over time. Associations will be adjusted for age, race, sex, energy intake, smoking status, presence of knee osteoarthritis, clinic assignment, level of physical activity, level of depression, and time between visits. A path diagram of the hypothesized relationship between fish intake and central adiposity is presented in Figure 1.

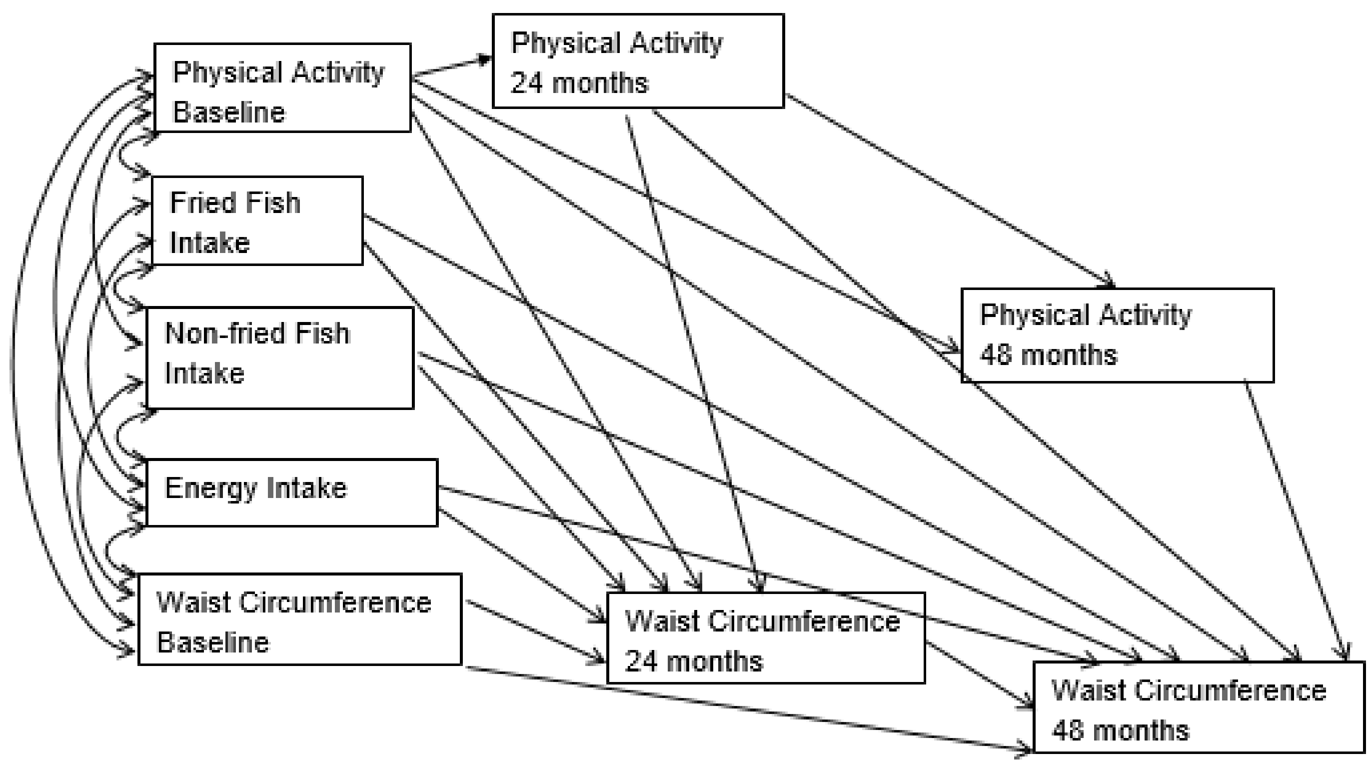

Figure 1. Path diagram for the hypothesized relationship between fish intake and waist circumference over time. Baseline covariates or factors include age, sex, race, smoking status, level of depression, presence of knee osteoarthritis, and time between visits.

Research Questions

What is the relationship between *non-fried fish intake* and central adiposity over time? What is the relationship between *fried fish intake* and central adiposity over time? How do age, race, sex, energy intake, smoking status, presence of knee osteoarthritis, clinic assignment, level of physical activity, level of depression, and time between visits mediate or moderate this relationship?

Hypotheses

When controlling for age, sex, race, presence of osteoarthritis, clinic assignment, smoking status, amount of fried fish eaten, energy intake, physical activity level, level of depression and time between visits, the amount of non-fried fish that a person consumes is negatively related to his or her waist circumference over time.

When controlling for age, sex, race, presence of osteoarthritis, clinic assignment, smoking status, amount of non-fried fish eaten, energy intake, physical activity level, level of depression, and time between visits, the amount of fried fish that a person consumes is positively related to his or her waist circumference over time.

Significance of the Problem

As a prevalent condition and important predictor of disease across race, gender and age groups, central adiposity is an important target in public health efforts to improve body composition in the general public. The American Heart Association (AHA) (Lichtenstein et al., 2006), the 2015-2020 Dietary Guidelines for Americans (Department of Health and Human Services & Agriculture, 2015), and the Academy of Nutrition and Dietetics (AND) (Vannice & Rasmussen, 2014) have all published recommendations that suggest approximately 8 ounces of fish should be consumed in the average diet each week, but no specific distinction is made between non-fried and fried fish is made in any of the recommendations. If the effect of fish intake on central adiposity is different for non-fried and fried fish, then dietary recommendations for fish intake should specifically recommend increasing non-fried fish and shellfish exclusively and warn against fried fish intake.

There is a lack of longitudinal research on the effects of whole fish in the diet and central adiposity, but there is evidence to support the hypothesis that fish intake is inversely related to waist circumference. This is the first known study to examine the longitudinal relationship between non-fried and fried fish intake and waist circumference across time.

CHAPTER TWO: REVIEW OF LITERATURE

There is evidence that increased intake of fish and shellfish may decrease prevalence and severity of central adiposity. This literature review will examine the relationships between dietary fish and fish oils and central adiposity. Additionally, the potential relationships between central adiposity and age, race, sex, energy intake, smoking status, presence of knee osteoarthritis, level of physical activity, level of depression, and time will be reviewed. Potential relationships between fish and fish oil intake and these control variables will also be discussed.

Purpose of the Current Study and Hypotheses

The purpose of the current prospective study is to investigate the longitudinal relationships between baseline fish intake and repeated measurements of waist circumference in a sample of 3,033 men and women. The repeated measurements of waist circumference at baseline, 24 months follow-up and 48 months follow-up will allow for investigation of associations between fish intake measured at baseline and change in waist circumference over time. Associations will be adjusted for age, race, sex, energy intake, smoking status, presence of knee osteoarthritis, clinic assignment, level of physical activity, level of depression, and time between visits.

When controlling for age, sex, race, presence of knee osteoarthritis, clinic assignment, smoking status, amount of fried fish eaten, energy intake, physical activity level, level of depression, and time between visits, the amount of non-fried fish that a person consumes is negatively related to his or her waist circumference over time.

When controlling for age, sex, race, presence of knee osteoarthritis, clinic assignment, smoking status, amount of non-fried fish eaten, energy intake, physical activity level, level of depression, and time between visits the amount of fried fish that a person consumes is positively related to his or her waist circumference over time.

Adipose Tissue and Obesity

An excess accumulation of body fat, or adipose tissue, is usually called obesity (Tchernof & Despres, 2013). Adipose tissue is a storage site for excess calories in the body, but it is also metabolically active organ that secretes multiple chemical signals into the blood that can target nearby tissues. These chemical signals may have an effect on food intake and metabolism, as well as pro- or anti-inflammatory effects in the body (Masoodi, Kuda, Rossmeisl, Flachs, & Kopecky, 2015). Excess adipose tissue allows an imbalance in chemical signals, setting the stage for inflammation and increased prevalence of chronic disease.

Obesity is significantly associated with mortality in both men and women (Adams et al., 2006). This association strengthens significantly with age (Masters et al., 2013). Between 1986 and 2006, excess weight is thought to have been responsible for almost 1 in 5 (18.2%) of deaths among white and black adults in the United States (Masters et al., 2013). Black women were found to have the highest percentage of deaths associated with excess weight (26.8%) followed by white women (21.7%), white men (15.6%) and then black men (5.0%) (Masters et al., 2013). Overweight and obesity were estimated to be accountable for almost 1 in 10 deaths in the United States, second only to smoking (Danaei et al., 2009). Obesity decreases life expectancy in adults, particularly when obesity is found in young adults compared to older adults (Fontaine, Redden, Wang, Westfall, & Allison, 2003).

Obesity is a risk factors for several chronic diseases, including insulin resistance, type 2 diabetes, and atherosclerosis (Hotamisligil, 2006). Obesity is an independent risk factor for hypertension, stroke, and coronary heart disease and is responsible for 12% of the preventable cardiovascular deaths each year in men and 7.4% in women (Patel et al., 2015). Obesity is strongly and positively related to type 2 diabetes with a relationship that gets stronger with age (Nkondjock & Receveur, 2003; Wang et al., 2005). Excess weight is associated with increased prevalence of several cancers, including uterine, gallbladder, kidney, liver and colon (Bhaskaran et al., 2014)

Obesity and its related health complications are estimated to cost the United States $147 billion dollars each year (Finkelstein, Trogdon, Cohen, & Dietz, 2009). From 1998 to 2011, mean annual per capita health care expenses were $1,809 higher for an obese American compared to a non-obese American (An, 2015). Health care expenses related to obesity were found to be higher among women, non-Hispanic whites and older adults compared to men, minority groups and younger adults, respectively (An, 2015). Obesity may be costly for obese individuals too, being obese was found to lower wages by 9% in women (Cawley, 2004).

Obesity is a prevalent concern. In 2011-2012, more than one third (34.9%) of American's over the age of 20 years were obese, 33.5% among men and 36.1% among women (Ogden, Carroll, Kit, & Flegal, 2014). Among US men and women between the ages of 45 to 79 years, obesity prevalence in 2009 - 2010 was 37.1% for men and 38.4% for women (Patel et al., 2015).

There are striking differences between major racial/ethnic groups on obesity prevalence. In 2011-2012, an estimated 47.8% of all non-Hispanic blacks over the age of 19 were obese, 42.5% of Hispanics, 32.6% of whites and only 10.8% of Asians (Ogden et al., 2014). Black women were found to have the highest prevalence of obesity at 56.6%, followed Hispanic women at 44.4% (Ogden et al., 2014). Obesity prevalence also varies by age. In 2011-2012, 30.3% of people 20 – 39 years were obese, 39.5% of people 40 – 59 years and 35.4% of people over 60 years (Ogden et al., 2014). This suggests that obesity is most prevalent in middle age. While increases in obesity prevalence in the general population has slowed in recent years (Flegal, Carroll, Kit, & Ogden, 2012), obesity prevalence in women over the age of 60 significantly increased in 2011-2012 (Ogden et al., 2014).

Understanding how obesity is defined and accessed is important in understanding the impact that excess adiposity has on an individual and also among the larger population (Rosito et al., 2008). There is a lack of consistency in the measurement of obesity in the health literature. The most accurate measurements of adipose tissues are evaluated in a laboratory setting, using methods such as computed tomography (CT), dual-energy X-ray absorptiometry (DEXA), and magnetic resonance imaging (MRI) but the use of anthropometric measurements, such as height, weight and waist circumference, have been found to be reliable indicators of obesity and disease risk status (Barreira et al., 2012).

Among anthropometric measures used to assess obesity, the most common measure is based on the body mass index (BMI). BMI is measured by taking an individual's weight in kilograms (kg) divided by height in squared meters (m^2). A BMI of 18.5 – 24.9 kg/m^2 is classified as healthy or normal weight, 25.0 – 29.9 kg/m^2 is overweight and a BMI of 30.0 or above is classified as obese (Centers for Disease Control and Prevention, 2012; Patel et al., 2015). Obesity can be further classified into class I (BMI 30.0 – 34.9 kg/m^2), class II (BMI 35.0 – 39.9 kg/m^2) and class III (BMI ≥ 40.0 kg/m^2). (Pi-Sunyer et al., 1998).

While BMI has been found to be reliable indicators for chronic disease risk, BMI is a flawed measurement for assessing adiposity. A meta-analysis of 25 studies looking at BMI performance found only a 0.50 pooled sensitivity for BMI to identify excess body adiposity (Okorodudu et al., 2010). An examination of the National Health and Nutrition Examination Survey (NHANES III) found that measuring obesity based on BMI failed to detect obesity in 13% of black men, 34% of black women, 32% of white men and 45% of white women compared to error rates closer to 3% when waist circumference was used to detect obesity in black women and black and white men (O'Neill, 2015). Waist circumference had a higher error rate than BMI (16% vs 13%) in black men.

BMI does not distinguish fat from muscle or bone nor can BMI distinguish between adipose tissue that collects in the abdominal region from adipose tissue that collects in the lower half of the body, such as on the hips and thighs. For these reasons, BMI is probably not the best anthropometric measure to use when assessing obesity risk.

Central Adiposity

Excess adipose tissue in the body tends to accumulate either as subcutaneous adipose tissue (SAT) or as visceral adipose tissue (VAT) (Hajer, van Haeften, & Visseren, 2008). SAT is found all over the body and provides physical protection, temperature control and energy storage. VAT is typically accumulated around the middle of the body and in the abdominal cavity. Excess accumulation of both types of adipose tissue increase prevalence of metabolic risk factors including hypertension and dyslipidemia, but compared to level of SAT, level of VAT is more strongly correlated to metabolic risk factors (Fox et al., 2007) and all-cause mortality (Katzmarzyk, Mire, & Bouchard, 2012).

An excess of VAT is called *central adiposity* (also called central or visceral obesity) (Shuster et al., 2012). VAT is believed to be more metabolically active than SAT and as a result has a stronger influence on blood glucose control and other hormonal actions in the body and poses a greater health risk (Bjorntorp, 1991; Fox et al., 2007; Katzmarzyk et al., 2013). Among obese individuals, metabolic risk factors increased significantly across increasing levels of VAT quartiles (Fox et al., 2007).

Prevalence estimates using BMI to define obesity suggest that more than one-third of US adults are obese, but it is believed that prevalence of central adiposity, measured by waist circumference, is even higher (O'Neill, 2015). The prevalence of central adiposity and the average waist circumference have both increased substantially since 1988 among US men and women (Ladabaum et al., 2014). Black women in the US have seen the greatest increase in prevalence of central adiposity. Central adiposity among normal-weight men and women has also increased. An examination of the 2007 – 2010 NHANES data found an average waist circumference of 95.2 cm in women over the age of 20 years and 100.9 cm in men over the age of 20 years (Fryar et al., 2012) This is considered an elevated waist circumference in women (> 88 cm), but just under the elevated level in men (> 102 cm). After the age of 50 years, all men had an average waist circumference > 102 cm. Waist circumference increased until the age of 70 years in women and until the age of 80 years in men.

A French physician is reported to be the first to publish results suggesting that fat accumulated in the abdominal area was more dangerous than fat accumulated in the lower body (Vague, 1956). Cohort studies from data collected in the late 1960s found that waist to hip circumference ratio was a strong predictor of the 12-year incidence of cardiovascular disease and mortality in women (Lapidus et al., 1984) and in men (Larsson et al., 1984). Recently, anthropometric measurements of central adiposity were found to be better predictors of cardiovascular risk compared to BMI (Kahn & Bullard, 2016).

Level of VAT and waist circumference have been found to be better predictors of cardiovascular risk factors than BMI, waist-to-hip ratio, and total body fat, but VAT did not perform significantly better than waist circumference (Barreira et al., 2012), therefore in cases where it is not reasonable to measure VAT, it is recommended that waist circumference be used to measure central adiposity (Katzmarzyk et al., 2013). Among US adults, the error rate for waist circumference measures of obesity was found to be approximately 3% compared with up to 45% error rate for BMI (O'Neill, 2015). Waist circumference is an independent risk factor for hypertension, diabetes, dyslipidemia and the metabolic syndrome (Barreira et al., 2012; Pouliot et al., 1994). The National Heart, Lung and Blood Institute (NHLBI) with the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) recommends the use of a patient’s BMI and waist circumference when assessing obesity risk status (Pi-Sunyer et al., 1998). An elevated waist circumference is indicated if > 102 cm (40 in.) in men and > 88 cm (35 in.) in women (Pi-Sunyer et al., 1998).

High levels of VAT may cause metabolic disturbances that are responsible for many of the metabolic issues seen in the obese, including type 2 diabetes and chronic low-grade inflammation (Hotamisligil, 2006; Tchernof & Despres, 2013). Central adiposity is positively correlated to CVD risks and negatively correlated with HDL cholesterol (Barreira et al., 2012). Elevated waist circumference is a significant predictor of risk factors for chronic disease and mortality, independent of BMI. Within BMI classes - including normal weight – having a high-risk waist circumference was associated with increased risk of hypertension, diabetes, dyslipidemia and metabolic syndrome compared to those with low-risk waist circumference status within the same BMI class (Janssen, Katzmarzyk, & Ross, 2002). Central adiposity for a given BMI is a strong indicator of risk of all-cause mortality among men and women (Bigaard et al., 2003; Katzmarzyk et al., 2012).

After insulin resistance, central adiposity is thought to be the predominant risk factor for metabolic syndrome. The National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) diagnostic recommendations for metabolic syndrome are the presence of three out of five of the following characteristics: (1) presence of central adiposity, typically measured as waist circumference, (2) elevated triglycerides, (3) reduced high-density lipoprotein cholesterol (HDL-C), (4) elevated blood pressure, and (5) elevated fasting glucose (Grundy et al., 2005).

Participants classified as obese according to BMI guidelines (BMI ≥ 30 kg/m^2) with the presence of central adiposity (waist circumference ≥ 88 cm in women and ≥ 102 cm in men) were found to have a 40% higher mortality risk compared to those classified as obese but without the presence of central adiposity (van der A et al., 2014). Having a healthier waist circumference (< 88 cm in women, < 102 cm in men) decreased risk of mortality by 14% compared to those with an elevated waist circumference (Behrens et al., 2013). Waist circumference was found to be a better predictor of mortality from specific causes than BMI (Leitzmann et al., 2011). Waist circumference is positively associated with mortality, even after adjustment for BMI (Carmienke et al., 2013).

Obese individuals clearly have an increased risk for comorbid conditions when compared to those of normal weight, but not all obese people present with comorbidities (Tchernof & Despres, 2013). Additionally, high risk for chronic disease is seen in individuals with a normal BMI but excess abdominal fat (Tchernof & Despres, 2013).

As a prevalent condition and important predictor of disease across race, gender and age groups, central adiposity is an important target in public health efforts to improve body composition in the general public. The effect of dietary intake, particularly energy intake, on body weight are generally well understood, but the effects of individual components of the diet are not as well understood and the results of dietary interventions have been mixed (Department of Health and Human Services & Agriculture, 2015; Katan & Ludwig, 2010).

Fish and Fish Oils

Fish, such as salmon and tuna, and shellfish, such as shrimp and oysters, are collectively are referred to as *seafood* (Department of Health and Human Services & Agriculture, 2015). *Fish oils* refer to the marine-derived long-chain omega-3 fatty acids (LC n-3 PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). They are called *marine-derived* because they are almost exclusively found in fish and shellfish and to a small extent in certain seaweeds (Vannice & Rasmussen, 2014). The beneficial effects of dietary fish intake are thought to be due in large part to the LC n-3 PUFA content of fish and shellfish (Mozaffarian & Wu, 2011), though there is evidence that fish protein has beneficial effects beyond the LC n-3 PUFA content (He, 2009).

Most dietary fat exists as triglycerides, a molecule made up of a glycerol backbone connected to three fatty acids. These individual fatty acids have different effects in the body (Kalish, Fallon, & Puder, 2012). Fatty acids are characterized by their degree of saturation, saturated fatty acids (SFAs) are fully saturated with no double bonds between the carbon atoms, monounsaturated fatty acids (MUFAs) have only one point of unsaturation, and polyunsaturated fatty acids (PUFAs) have two or more points of unsaturation (Kalish et al., 2012). PUFAs are oils and like all fatty acids, PUFAs can be oxidized for energy, incorporated into plasma phospholipids and cell membranes, and serve as mediators for chemical messengers, such as eicosanoids (Kalish et al., 2012).

PUFAs can be subdivided into groups according to the location of the first point of unsaturation. The omega-3 PUFAs (n-3 PUFA) have the first point of unsaturation at the third carbon from the omega end. In 2005, low dietary intake of n-3 PUFA was accountable for 84,000 or 3.4% of deaths in the United States (Danaei et al., 2009). The three most prominent n-3 PUFAs are *alpha*-linolenic acid (ALA), EPA, and DHA.

ALA is the 18-carbon n-3 PUFA and is the most consumed n-3 PUFA in the typical American diet (Burdge & Calder, 2005; Vannice & Rasmussen, 2014). ALA is considered the *plant-derived* n-3 PUFA and is found in flaxseed, beans, leafy green vegetables, and nuts (Burdge & Calder, 2005). EPA is the 20-carbon n-3 PUFA and DHA is the 22-carbon n-3 PUFA, they are considered the marine-derived n-3 PUFA because they are almost exclusively found in fish and shellfish (Vannice & Rasmussen, 2014). When EPA and DHA are discussed together they are often referred to as fish oils.

Fish and general health.

Dietary intake of fish became a topic of interest in health research after populations with higher than average fish and seafood intake were observed to have a low prevalence of cardiovascular disease (Bang et al., 1980; Middaugh, 1990). The potential benefits of fish oil supplementation were first examined in paper published in 1966. The authors found that plasma triglycerides were reduced after administration of fish oil (Engelberg). Since that time, the body of evidence to support the triglyceride lowering effects of LC n-3 PUFA have become well established, to the point that a prescription form of LC n-3 PUFA has been available to treat hypertriglycemia since 2004 (He, 2009).

Increasing intake of LC n-3 PUFAs is largely accepted as an effective dietary strategy to reduce prevalence of cardiovascular disease risks (Hu & Willett, 2002). Increased dietary intake of fish and shellfish is associated with reduced prevalence of hypertension (Ke et al., 2014), hypertriglyceridemia (Itariu et al., 2012), all-cause mortality, ischemic heart disease and stroke (Zhang, Sasaki, Amano, & Kesteloot, 1999). Increased intake of LC n-3 PUFAs in the form of a fish oil supplement can reduce blood pressure, total cholesterol and triglyceride levels compared to control group (Ebrahimi et al., 2009). Consuming fish at least once per week was associated with reduced prevalence of sudden cardiac death in men (Albert et al., 1998). Fish and seafood intake has been associated with reduced risk of type 2 diabetes in populations with high prevalence of obesity (Nkondjock & Receveur, 2003). LC n-3 PUFA status has been found to be negatively associated with all-cause mortality in men and women (Marklund et al., 2015; Nagata, Takatsuka, & Shimizu, 2002).

Fish intake may be related to metabolic syndrome. Men who ate fish almost daily had a significantly lower prevalence of metabolic syndrome compared to those who ate fish less than once per week (Baik, Abbott, Curb, & Shin, 2010). There was no effect seen in women. Other studies have also found a significant inverse associate between fish intake and metabolic syndrome in men only and not in women (Kouki et al., 2011). Among Iranian woman, those in the highest tertile for fish intake were 65% less likely than those in the lowest tertile to have metabolic syndrome (Zaribaf et al., 2014). For each additional serving of fish eaten each week, incidence of metabolic syndrome was reduced by 6% and for every 100 mg/day increment in LC n-3 PUFA intake incidence were reduced by 12%. (Kim, Xun, & He, 2015). Not all studies have found this association (Lai et al., 2013).

A meta-analysis examining LC n-3 PUFA intake and prevalence of stroke morbidity and mortality found that higher LC n-3 PUFA intake was negatively related to both stroke morbidity and mortality in women, but no significant effects were seen in men (Cheng et al., 2015). Other studies have also found stronger relationships with LC n-3 PUFA and cardiovascular risk factors in women compared to men (Marklund et al., 2015).

EPA and DHA may have independent effects on cardiovascular risk factors and DHA may be more important that EPA in increasing HDL cholesterol and reducing blood pressure (Mori & Woodman, 2006). It may be there EPA and DHA have different effects on the functioning of adipose cells (Oster, Tishinsky, Yuan, & Robinson, 2010).

In addition to the beneficial relationship between fish intake and cardiometabolic risk factors, higher levels of LC n-3 PUFA intake may have a number of additional beneficial effects on human health. Higher intake of fish and fish oil were found to be significantly associated with fewer depressive symptoms (Hoffmire, Block, Thevenet-Morrison, & van Wijngaarden, 2012) and lower levels of depression in older adults (Skarupski, Tangney, Li, Evans, & Morris, 2013). Eating baked or broiled fish on a weekly basis was related to larger volume in areas of the brains responsible for memory in cognitively normal elderly individuals. This effect was independent of the plasma levels LC n-3 PUFA. (Raji et al., 2014). Fish intake may be related to lower incidence of cancer (Turunen et al., 2014). Fish intake was positively associated with improved kidney function in men and women over the age of 65 years (Chrysohoou et al., 2013)

Current recommendations.

Predominantly based on the strength of the evidence of a beneficial relationship with heart disease, the current general recommendation for healthy US adults is to consume fish at least twice per week, consuming approximately 8 ounces per week or approximately 250 mg per day of LC n-3 PUFA (Department of Health and Human Services & Agriculture, 2015).

The American Heart Association (AHA) recommends consuming a 4 ounce serving of fish, particularly oily fish, (the equivalent of ¾ cup of flaked fish such as salmon or tuna) at least two times per week to reduce risk of cardiovascular disease (Lichtenstein et al., 2006). The authors of the AHA statement suggest that in addition to the EPA and DHA found in fatty fish, increasing fish intake can displace other, less healthful types of fat in the diet (saturated fat and *trans* fats).

The Academy of Nutrition and Dietetics (AND) specifically recommends a dietary, food-based approach to increasing LC n-3 PUFA by consuming two or more servings of fatty fish per week, the equivalent of at least 500 mg EPA and DHA each day (Vannice & Rasmussen, 2014). Additionally, AND recommends 20% to 35% of daily energy should come from fat with increased LC n-3 PUFA and minimal saturated and *trans* fats.

Americans generally do not eat the recommended amount of fish each week. The NHANES collects information about frequency of fish and shellfish intake in the past 30 days. Examination of the mean intakes among US adults found that most are not eating the recommended 7-8 ounces of fish each week (Papanikolaou et al., 2014). Overall fish intake for all adults was 0.61 ounces/day but it intake dropped to only 0.15 ounces/day when looking exclusively at high LC n-3 PUFA fish. Men eat more fish than women (mean usual intake of 0.71 ounces/day vs. 0.51 ounces/day) and older adults eat more fish than younger adults (0.66 ounces/day vs. 0.58 ounces/day).

Assessing fish intake.

Because EPA and DHA are scarce in foods sources other than fish and shellfish, habitual fish and fish oil intake can be estimated through dietary questionnaires, such as food frequency questionnaires (FFQs), which have been found to be valid for estimating fish intake (Garneau et al., 2012; Ingram, Stonehouse, Russell, Meyer, & Kruger, 2012) or through biomarkers for LC n-3 PUFA status, such as profiles of plasma phospholipid fatty acid content (Howe & Buckley, 2014).

FFQs are simple and low-cost tools to examine habitual fish and seafood intake and total n-3 PUFA intake. FFQs ask about how often and how much of selected foods or nutrients are eaten. Evidence of validity and reliability of FFQs for assessing EPA, DHA, and total LC n-3 PUFA in the diet has been found using erythrocyte LC n-3 PUFA content as the standard (Ingram et al., 2012; Mina, Fritschi, & Knuiman, 2007) and using plasma phospholipid LC n-3 PUFA content as the standard (Garneau et al., 2012; McNaughton, Hughes, & Marks, 2007).

Surrogate biomarkers of LC n-3 PUFA intake can also be used to estimate LC n-3 PUFA status. Plasma fatty acid content can detect recent LC n-3 PUFA intake (days), relative levels found in plasma phospholipids can be used to estimate intake over a period of weeks and looking at the relative content of LC n-3 PUFA in erythrocytes can be used to examine longer-term intake (Howe & Buckley, 2014).

Biomarkers of LC n-3 PUFA can also be used to estimate fish intake. ALA can be converted endogenously into EPA and DHA, but the rate is limited to less than 10% in humans, therefore levels of LC n-3 PUFA in blood and tissue are predominantly determined by dietary intake (Burdge & Calder, 2005). Fish intake was strongly correlated to plasma phospholipid LC n-3 PUFA ($r = 0.78$, $p < 0.01$) (Saadatian-Elahi et al., 2009). Biomarkers of DHA status have been found to be better correlated to fish intake than biomarkers of EPA status (Baylin, Kabagambe, Siles, & Campos, 2002). To estimate risk of low LC n-3 PUFA intake, it may be most important to examine DHA status.

LC n-3 PUFA are considered essential fats because the body cannot make then in quantities high enough to support health, so they must be obtained from the diet.

Additional benefits of fish protein.

Ideally, nutritional needs should be fulfilled by food as opposed to supplements. The 2015 *Dietary Guidelines* state that the recommendation to eat at least 8 ounces of fish and shellfish weekly reflect the need for all of the nutrients that fish and shellfish can provide, including EPA and DHA (Department of Health and Human Services & Agriculture, 2015). The effects of dietary fish intake are often assumed to be comparable to the effects of supplemental fish oil intake (Bender et al., 2014), but there is evidence that components of fish protein beyond EPA and DHA may be additionally beneficial for health (He, 2009). Fish protein contains additional nutrients beyond the LC n-3 PUFA content, including taurine, arginine, glutamine, selenium and calcium, vitamin D, and vitamin B. Taurine is an amino acid that is abundant in fish protein that has been associated with weight loss (Zhang et al., 2004) and reduced inflammation (Elvevoll et al., 2008). Benefits to cognitive function were found to be related to weekly fish intake, independent of the LC n-3 PUFA content of the diet (Raji et al., 2014).

LC n-3 PUFA from fish protein may be better at increasing biological levels of EPA and DHA compared to fish oil supplements. After 8 weeks, serum levels of EPA and DHA were significantly higher in the fatty fish group compared to fish oil group, even though the fish oil group consumed 3 times as much LC n-3 PUFA (Elvevoll et al., 2006). Not all evidence suggest that fish protein is superior to fish oil supplements. Fish oil interventions have the advantage of being able to continuously administer EPA + DHA to participants in the form of fish oil capsules and daily intake of fish oil was observed to lead to greater incorporation of EPA + DHA in body tissues compared to twice weekly administration of an equivalent amount of EPA + DHA (Browning et al., 2014). There was no difference observed between blood lipid levels of EPA + DHA of women who ate fatty fish weekly compared to women who took daily fish oil supplements (Harris, Pottala, Sands, & Jones, 2007).

Lean fish, such as cod, is typically low in LC n-3 PUFA content, but lean fish and fatty fish have been found to have similar, beneficial effects on weight loss and waist circumference (Ramel, Jonsdottir, & Thorsdottir, 2009; Thorsdottir et al., 2007) and on levels of insulin resistance (Ouellet, Marois, Weisnagel, & Jacques, 2007) and markers of inflammation (Ouellet et al., 2008). Lean fish has been found to decrease body fat and improve glucose control compared to control group (Vikoren, Nygard, Lied, Rostrup, & Gudbrandsen, 2013).

Some of the health benefits seen with increased fish intake could be due to the replacement of bad fats, such as saturated and *trans* fats, in the diet. Lower-fat proteins, like fish and shellfish, can replace higher-fat proteins, like red meat, in the diet (Lichtenstein et al., 2006). In addition to being overall lower in fat, fish protein typically has a healthier profile of fatty acids than red meat or poultry (P. Howe, Meyer, Record, & Baghurst, 2006). Those with the highest levels of fish intake have been found to have the highest levels of vegetable, fruit and berry intake and the lowest levels of red meat and sausage intake (Turunen, Mannisto, Suominen, Tiittanen, & Verkasalo, 2011).

Effects of cooking methods.

None of the current dietary recommendations for fish and shellfish intake make a specific distinction between non-fried and fried fish. The way that fish is cooked may affect how much of the EPA and DHA is retained in the fish protein and may be an important consideration in dietary recommendations for fish and shellfish intake.

Frying tuna fish was found to destroy 70% of the EPA and 85% of the DHA and canning tuna fish has been found to destroy all of the EPA and DHA found in raw tuna fish (Stephen, Shakila, Jeyasekaran, & Sukumar, 2010). Cooking the fish in a boiling water bath and microwaving for short periods did not significantly destroy the EPA or DHA. Neff looked at the effect of frying Chinook salmon, common carp, lake trout and walleye and found that frying, baking and broiling the fish did not significant lower the EPA or DHA content (Neff, Bhavsar, Braekevelt, & Arts, 2014). They did find that frying fish decreased the n-3 to n-6 ratio of oil in the fish, due to n-6 PUFA absorbed from the cooking oil. Decreased n-3 to n-6 ratio is associated with adverse health outcomes (Vannice & Rasmussen, 2014).

In addition to the loss LC n-3 PUFA content, cooking methods like frying fish, particularly deep frying, may add to the caloric and saturated fat content of the food. Fried fish has been linked to increased CHD, but not non-fried fish. Breaded fish was related to increased risk of higher severity of depressive symptoms but no relationship was found for non-breaded fish (Hoffmire et al., 2012).

Environmental contaminants.

When examining dietary fish and shellfish, concerns about environmental contaminants should be discussed. While special populations, such as children and pregnant women should be mindful of mercury contamination that is possible in fish and shellfish, the potential benefits of fish intake outweigh potential contamination risk for middle age or older men and postmenopausal women (Lichtenstein et al., 2006; Mozaffarian & Rimm, 2006). It is suggested that most dietary seafood choices be low in methyl mercury but high in LC n-3 PUFA, such as salmon, anchovies, sardines, trout and Pacific oysters, to limit exposure to mercury (Department of Health and Human Services & Agriculture, 2015).

Fisherman and their families in Finland, believe to consume at least 1.5 times as much fish as the general population, were found to have the same total cancer incidence as the general population (Turunen et al., 2014), suggesting that the level of potentially cancer-causing environmental contaminants that are found in fish does not increase cancer prevalence, even among those with high fish intake.

Increasing levels of EPA and DHA in the diet, either through eating fish protein or taking fish oil supplements, may have a beneficial effect on health, including central adiposity.

Fish and Central Adiposity

There is evidence that increased fish and fish oil intake may have a beneficial relationship with general obesity and central adiposity (Bender et al., 2014; Panagiotakos, Pitsavos, Skoumas, & Stefanadis, 2007; Ramel et al., 2009; Ruzickova et al., 2004; Thorsdottir et al., 2007). The 2015-2020 Dietary Guidelines for Americans state there is moderate evidence that diets that include seafood are associated with decreased prevalence of obesity (Department of Health and Human Services & Agriculture, 2015). The first published meta-analysis of fish and fish oil intake and body composition supports the hypothesis that fish intake is associated with obesity. The authors found a significant difference between treatment and control groups on the outcomes of body weight lost (-0.59 kg more lost than control), reduced BMI (-0.24 kg/m^2), decreased percent body fat (-0.49%) and reduced waist circumference(-0.81 cm) (Bender et al., 2014).

A Mediterranean style diet is typically higher in fish and seafood intake compared to more Western diets and has been associated with lower waist circumference and other measures of obesity. A meta-analysis of 50 studies found that adherence to the Mediterranean diet was associated with decreased waist circumference (-0.42 cm) compared to control diet (Kastorini et al., 2011).

Couet was one of the first to publish on the intervention effects of fish oil on body composition. Six healthy adults followed a control diet, ad libitum, for 3 weeks, then a 10 to 12 weeks wash-out period, and then they followed the same control diet with 6 g of visible fat in the diet replaced with 6 g of fish oil (1.1 g/day EPA and 0.7 g/day DHA). Body weight was unchanged but body fat mass was significantly reduce during the intervention period compared to the control diet (Couet, Delarue, Ritz, Antoine, & Lamisse, 1997).

Participants who consumed a fish protein supplement (made from cod) for 4 weeks, with no change to their typical diet or exercise routine, saw a significant decrease in percent body fat compared to baseline values (Vikoren et al., 2013). After 8 weeks in this trial, the fish protein supplement group had significantly lower fasting glucose levels compared to control group.

Twelve weeks of supplementing with 6 g tuna fish oil per day (approximately 1.6 g/day DHA and 0.4 g/day EPA) significantly reduced fat mass among overweight adults compared to control group. (Hill, Buckley, Murphy, & Howe, 2007). Obese individuals have been found to have significantly lower plasma levels of LC n-3 PUFA compared to those with a healthy weight status (Micallef, 2009). The relationship between fish and fish oil intake and obesity may be stronger in the obese population compared to the overweight and healthy weight population. Among 124 middle-aged men and women (mean age = 49.5 years), stratified by weight status according to BMI (healthy weight, overweight and obese), there were significant negative associations between plasma EPA and DHA levels and BMI (r = -0.32 and -0.36 respectively), waist circumference (r = -0.24 and -0.28) and hip circumference (r = -0.32 and -0.38) in the obese group only, there were no significant associations found in the healthy weight and overweight group (Micallef, Munro, Phang, & Garg, 2009). The authors additionally pooled all the weight status groups and stratified participants into quartiles according to total LC n-3 PUFA plasma concentration and found significant inverse trends with BMI, waist circumference and hip circumference.

Several clinical studies indicate that increased LC n-3 PUFA intake can enhance the amount of weight lost when combine with an energy-restricted diet compared to an energy-restricted diet alone (Kabir et al., 2007; Munro & Garg, 2013), but other studies have not found any additional benefit from increasing LC n-3 PUFA with an energy-restricted diet (Crochemore, Souza, de Souza, & Rosado, 2012; Munro & Garg, 2012).

Two months of LC n-3PUFA supplementation (1.8 g/day) in 26 women with type 2 diabetes was found to significantly decrease fat mass compared to placebo. They found no change in body weight or energy intake between the groups (Kabir et al., 2007). Increasing PUFA intake in the diet has been associated with decreased levels of abdominal fat and increased insulin sensitivity (Summers et al., 2002).

There is evidence that the effect of fish intake on obesity may be stronger in men than in women. Men who consumed cod or salmon or fish oil supplements while on an energy-restricted diet lost significantly more weight and had a greater reduction in waist circumference compared to control group, but no significant differences were seen among women (Thorsdottir et al., 2007). Among the 43,671 men in the Health Professional Follow-up Study, those with low fish intake were more likely to be overweight than were those with high fish intake (He et al., 2002).

Not all study results support an effect of fish intake on obesity. Eating fatty fish 3 times per week did not lead to greater weight loss compared to hypocaloric diets that were either high-protein or legume-based (Abete, Parra, & Martinez, 2009). Compared to a control group, adding 180 g of fatty fish per week or 420 mg EPA + 210 mg DHA fish oil supplement per day to low-calorie diet did not result in more body weight lost, reduced BMI or reduced percent body fat among middle aged obese adults (Tapsell et al., 2013). It should be noted that the authors reported that only 48% of the fish intake group was compliant to the recommendation and that 180 g of fish per week and 630 mg fish oil per day are low doses. Trials that included higher levels of fish intake have found relationships between fish intake and greater weight loss and healthier body composition (Ramel et al., 2009; Thorsdottir et al., 2007).

Among 26 men and women with a BMI between 28-33 kg/m^2 (overweight or moderately obese) no difference was observed in the amount of body weight lost by the LC n-3PUFA intervention group and the control group. The authors also reported no association with total calorie intake or appetite (Kratz, Callahan, Yang, Matthys, & Weigle, 2009).

There is very little evidence to support a detrimental relationship between fish intake and obesity, but positive associations have been found. Among the 79,839 women in the Nurses' Health Study higher intake of fish and LC n-3 PUFA were positively related to obesity status measured by BMI (Iso et al., 2001). A weak but positive association was found between total fish intake, as well as lean fish and fatty fish intake separately, and annual change in body weight (Jakobsen et al., 2013).

Beneficial relationship with central adiposity.

Results of associational investigations have found an inverse relationship between fish and fish oil intake and central adiposity. A diet pattern high in fish intake had a significant negative association with waist circumference (-0.88 cm) compared to dietary patterns that did not include fish (Panagiotakos et al., 2007). Lund and colleagues found significant inverse associations between total LC n-3 PUFA intake (derived from FFQ) and waist circumference (2013). Among a sample of the European Prospective Investigation in Cancer and Nutrition (EPIC) study, researchers examining associations between fish intake and waist circumference found a weak, negative association between fatty fish intake and change in waist circumference for all participants, but no association for lean fish (Jakobsen et al., 2012).

Among the 2005 and 2008 Framingham study participants, an inverse association was found between erythrocyte levels of EPA and DHA and waist circumference and BMI (Harris et al., 2012). The associations were found for EPA and DHA individually. In contrast, plasma phospholipid levels of DHA were found to be negatively correlated to waist circumference and BMI, but no relationship was found with EPA levels (Garneau et al., 2013).

There is evidence that the association between fish and waist circumference is stronger in women compared to men. Erythrocyte levels of EPA + DHA – indicating higher habitual intake – were inversely associated with waist circumference in women only, the relationship was particularly strong for DHA

(Howe et al., 2014). While no effect was found for waist circumference, supplementing a very low calorie diet with 6 g/day of fish oil was found to significant decrease the amount of weight lost for females in the fish oil group compared to females in the diet only group, but no difference was observed between men (Munro & Garg, 2013).

The relationship between fish and fish oil intake and central adiposity may be stronger in the obese population compared to the overweight and healthy weight population. The relationship between plasma levels of LC n-3 PUFA and weight status in 124 middle-aged men and women (mean age = 49.5 years), was examined, stratified by weight status according to BMI (healthy weight, overweight and obese) (Micallef et al., 2009). They found significant negative associations between plasma EPA and DHA levels and waist circumference ($r = -0.24$ and -0.28) in the obese group only, there were no significant associations found in the healthy weight and overweight group. They additionally pooled all the weight status groups and stratified participants into quartiles according to total LC n-3 PUFA plasma concentration and found significant inverse trends with waist circumference (Micallef et al., 2009).

There is also experimental evidence to support the hypothesis that higher fish and fish oil intake has a beneficial effect on adiposity. Participants who ate 5.3 ounces of cod per week along with an energy-restricted diet had a decrease in waist circumference of 3.4 cm more than those on the energy-restricted diet alone (Ramel et al., 2009). Cod is a lean fish, suggesting that it might be something in fish protein beyond just LC n-3PUFA that has an effect on decreased waist circumference.

Supplementation of 1.5 g/day of fish oil (328.5 mg EPA and 211.5 mg DHA) for 30 days was found to significantly reduce waist circumference compared to control group as well as compared to the 2.5 g/day of fish oil supplementation group (Crochemore et al., 2012). A diet high in complex carbohydrates and supplemented with 1.24 g/day of LC n-3 PUFA supplement reduced prevalence of enlarged waist circumference compared to equivalent diet without fish oil supplement (Paniagua et al., 2011).

There is evidence from intervention studies that the effect of fish on waist circumference may be stronger in men compared to women. Among 278 men, those who consumed 150 g of cod, 3 times each week (approximately 0.3 g/day of LC n-3 PUFA) or 150 g of salmon, 3 times each week (approximately 3.0 g/day of LC n-3 PUFA) or fish oil supplements (approximately 1.5 g/day LC n-3 PUFA) along with an energy restricted diet for eight weeks lost significantly more weight and had a greater reduction in waist circumference compared to a control group (Thorsdottir et al., 2007). These results were not seen among women.

No relationship with central adiposity.

The results of some investigations have not found a relationship between fish and fish oil intake and central adiposity. No association was found between fish intake and 5- year difference in waist circumference for men or women (Halkjaer, Tjonneland, Overvad, & Sorensen, 2009). Among adolescent boys and girls, there were no significant associations found between erythrocyte DHA and waist circumference (Lauritzen et al., 2012).

No significant effects on waist circumference were seen after 12 weeks of 1 g/day fresh salmon or equivalent fish oil (Brazionis, Ting, Itsiopoulos, Wilson, & Hodge, 2012). Among obese men and women, administration of 6 g fish oil per day in combination with a very low calorie diet had no effect on waist circumference compared to diet only group. There was a significant difference in the amount of weight lost between females in the fish oil group compared to females in the diet only group, but no difference was observed between men (Munro & Garg, 2013).

Obese women on an energy-restricted diet who received a supplement of 2.8 g/day fish oil were compared to a control group on the same diet without fish oil supplement. No difference between groups was observed for decrease in waist circumference, but those taking the fish oil supplement experienced greater BMI loss compared to control group (Kunesova et al., 2006). A moderately strong correlation was found between plasma levels of DHA and decrease in BMI ($r = -0.595$, $p = 0.008$), but not for EPA, suggesting that DHA may be responsible for decreased weight loss in obese women. Among overweight, insulin-resistant women, 5 g/day of fish oil (1.3 g EPA + 2.9 g DHA) added to a weight loss diet had no effect on waist circumference compared to those on the diet only (Krebs et al., 2006).

Among overweight and obese adults, 3 g/day EPA + DHA (5:1 ratio) in addition to dietary and exercise intervention over a 6 month period, did not have an effect on waist circumference compared to control (DeFina, Marcoux, Devers, Cleaver, & Willis, 2011). It should be noted that this study used a low-DHA content supplement and several studies have found stronger effects from DHA compared to EPA.

Itoh et al treated obese Japanese adults with diet therapy and 1.8 g/day of EPA only supplement. They found no effect on BMI or waist circumference compared to adults treated with diet therapy only (Itoh et al., 2007).

Women on an energy-restricted diet treated with 1.3 g/day EPA only supplement had no effect on change in body weight and no effect on change in waist circumference compared to control (Huerta, Navas-Carretero, Prieto-Hontoria, Martinez, & Moreno-Aliaga, 2015).

Detrimental relationship with central adiposity.

There are very few studies demonstrating a positive or detrimental effect of fish intake on central adiposity. A cross-sectional analysis of 1152 adults found positive associations between intake of fish and shellfish and abdominal obesity measured by waist circumference, but they did not differentiate between non-fried and fried fish (Alkerwi et al., 2015).

While research results are clearly mixed, there is evidence that fish and fish oil intake can have a beneficial effect on obesity/body composition and that one of the consequences of low fish intake might be increased risk of central adiposity.

Proposed Mechanisms

Potential mechanisms for the effect of fish and LC n-3 PUFA intake on central adiposity are not well understood, but there is evidence to support several possible mechanisms of effect. Decreased inflammation levels, beneficial fat metabolism, and beneficial correlations with other healthy behaviors are possible mechanisms.

Low-grade chronic inflammation is thought to be largely triggered by metabolic activity in adipose tissue and in particular VAT (Hotamisligil, 2006; Masoodi et al., 2015). Different types of fatty acids can serve as precursors for chemical messengers, such as eicosanoids, in the body. Increased LC n-3 PUFA in cell membranes modulates eicosanoid production toward a mostly anti-inflammatory state, lowering overall level of inflammation in the body (Kalish et al., 2012; Masoodi et al., 2015) and potentially lowering waist circumference (Festa et al., 2001). The positive effects seen with increased LC n-3 PUFA intake may be predominantly due to suppression of pro-inflammatory prostaglandins and leukotrienes (Kalish et al., 2012).

Circulating levels of adiponectin, an anti-inflammatory hormone secreted by adipose tissue, are lower in obese people compared to normal weight people. Decreased adiponectin levels are associated with increased cardiovascular disease risks and increased insulin resistance (Hui, Lam, Vanhoutte, & Xu, 2012). It is possible that increasing plasma levels of adiponectin reduces inflammation, which reduces body fat and waist circumference (Hui et al., 2012; Luft et al., 2013). LC n-3 PUFA intake has been found to have a positive relationship with adiponectin (Gray, Steyn, Davies, & Vitetta, 2013; Krebs et al., 2006) and a negative relationship with high sensitivity C-reactive protein, a marker for inflammation (Micallef, Munro, & Garg, 2009). Interventions using cod protein (Ouellet et al., 2008) and 3.36 g fish oil per day (Itariu et al., 2012) have found decreases in markers of inflammation compared to control group. It has been suggested that increased adiponectin levels decrease appetite (Howe & Buckley, 2014). Increased LC n-3 PUFA intake may beneficially impact the expression of genes that are responsible for fat metabolism, encouraging fatty acid oxidation and discouraging lipogenesis in adipose tissue which leads to decreased fat storage (Couet, et al., 1997; Howe & Buckley, 2014).

Additionally, there is evidence that increased intake of LC n-3 PUFA decreases appetite (Huerta et al., 2015; Krebs et al., 2006; Parra et al., 2008) and energy intake (Harden et al., 2014), which could lead to a healthier waist circumference. Increased fish and LC n-3 PUFA intake may increase compliance to an energy restricted diet by controlling hunger.

Other Effects on Central Adiposity to Consider

There are a number of other variables to consider when looking at the effects of fish intake on waist circumference. There is substantial evidence to support sex and age variations in waist circumference and some evidence for ethnic differences as well (World Health Organization, 2011).

Women were at greater risk of central adiposity than men, women were six times more likely to develop central adiposity than men (Leandro-Merhi, de Aquino, de Camargo, & de Oliveira, 2013). One advantage that waist circumference has over BMI is that gender-specific cut-offs are used to clinically identify a measure for obesity. It may be that sex-specific models are the most appropriate choices when using waist circumference as a measure of central adiposity. Women were found to gain significantly more weight over a 15-year period than did men (4.8 kg vs. 3.2 kg) and have significantly more increased waist circumference than did men (3.67 cm vs. 1.94 cm) (Arabshahi, Lahmann, Williams, & van der Pols, 2014). This study also found that women continue to gain weight and waist circumference longer into old age compared to men, who tend to have decreased waist circumference after about the age of 70 years (Fox et al., 2007).

It is becoming clear that gender differences exist on LC n-3PUFA status in adults. Plasma phospholipid LC n-3 PUFA were found to be significantly higher in women compared to men (Welch et al., 2006). Adolescent girls were found to have significantly higher erythrocyte DHA than boys (Lauritzen et al., 2012). An examination of erythrocyte levels of EPA and DHA found similar levels in men and women but EPA + DHA slightly lower in men than in women. Both DHA and EPA + DHA were found to be inversely correlated to BMI, waist circumference and body fat in women only (Howe et al., 2014). While no significant effects were found in men, women who followed an energy-restricted diet and consumed approximately 2 g LC n-3 PUFA per day lost significantly more weight and had a significantly reduced BMI compared to women following the same diet but consuming placebo oil (Munro & Garg, 2013). Serum levels of EPA and DHA were associated with lower CVD risk in women only (Marklund et al., 2015). Female sex was associated higher LC n-3 PUFA status (Harris et al., 2012). After 12 months of equivalent LC n-3 PUFA supplementation, women had significantly higher levels of plasma EPA compared to men (Walker et al., 2014).

Some results support a greater effect of LC n-3 PUFA in men compared to women. A stronger effect of LC n3-PUFA on reduced waist circumference was seen in men compared to women in the first meta-analysis of fish and fish oil intake and body composition (Bender et al., 2014). Men who consumed cod or salmon or fish oil supplements while on an energy-restricted diet (but no change to physical activity) lost significantly more weight and had a larger reduction in waist circumference compared to the control group, but no significant differences were seen among women (Thorsdottir et al., 2007). Men may be able to convert more ALA into DHA endogenously, compared to women (Burdge & Calder, 2005).

Though neither gender was found to consume the recommended 7-8 ounces of fish each week, men were found to consume more total fish as well as more fish high in LC n-3 PUFA than do women (Papanikolaou et al., 2014).

Waist circumference increases with age up until the age of 70 years in women and until the age of 80 years in men (Ford, Mokdad, & Giles, 2003; Fryar et al., 2012). The prevalence of abdominal adiposity was found to increase with age (Ladabaum et al., 2014). Age was associated with weight change over time in both men and women, but did not reach statistical significant for waist circumference change over time in women over a period of 15 years (Arabshahi et al., 2014).

Waist circumference has been found to increase over time in both men and women. (Jackson et al., 2015). Waist circumference may increase faster in women then in men. Among 22,570 women and 20,126 men, the median observed change in 5 year difference in waist circumference was 6.1 cm in women and 2.7 cm in men among the men (Halkjaer et al., 2009).

Age may have an effect on the amount of LC n-3 PUFA that is utilized by the body. After 12 months of equivalent LC n-3 PUFA supplementation, increases in plasma levels of EPA concentration were observed to increase with age, while increases in DHA concentration decreased with age (Walker et al., 2014).

Adults over the age of 51 years eat more total fish and more fish high in omega-3 fatty acids than do adults between the ages of 19 and 50 years, though neither age group consumed the recommended 7 to 8 ounces of fish per week (Papanikolaou et al., 2014).

Race and ethnicity may have an effect on prevalence of central adiposity. An examination of NHANES data from 1988 to 2010 found average waist circumference was largest among black women and smallest among white women, but among men, whites had the largest waist circumferences and blacks had the smallest (Ladabaum et al., 2014).

It would be expected that average energy intake would have a relationship with central adiposity, it is well understood that excess energy intake leads to weight gain (Katan & Ludwig, 2010), but average energy intake is not always observed to be associated with central adiposity (Arabshahi et al., 2014; Ladabaum et al., 2014; Leandro-Merhi et al., 2013).

Level of physical activity may also be associated with central adiposity. Among US men and women, increases in waist circumference were associated with level of physical activity but not with energy intake (Ladabaum et al., 2014). Level of physical activity was not associated with change in waist circumference over a 15 year period in men but it was found to have a significant effect on waist circumference in women (Arabshahi et al., 2014). A moderate exercise program was found to significantly decrease waist circumference (-0.57 cm) in postmenopausal women compared to a control group (Velthuis, Schuit, Peeters, & Monninkhof, 2009). Elevated waist circumference is associated with decreased physical function in adults of years of age (Batsis, Zbehlik, Barre, Mackenzie, & Bartels, 2014).

The effects of fish and fish oil may differ based on level of physical activity. Among healthy adults ages 30 – 54 years, biomarkers of LC n-3 PUFA status were negatively associated with cardiometabolic risk factors, including elevated waist circumference, but only among those who engaged in regular physical activity compared to inactive adults (Muldoon et al., 2013)

Elevated waist circumference was positively associated with major depressive symptoms as well as more moderate depressive symptoms (Zhao et al., 2011). Smoking status may also be related to central adiposity, light smoking is associated with decreased waist circumference but heavy smoking is associated with increased waist circumference (Chiolero, Faeh, Paccaud, & Cornuz, 2008). Presence of chronic disease, including osteoarthritis, may increase prevalence of central adiposity. Waist circumference has been found to be a strong predictor of knee osteoarthritis, independent of BMI (Janssen & Mark, 2006)

Summary

Weight loss is an important public health target. Reducing incidence and severity of obesity is one strategy for reducing the burden of chronic disease on the US healthcare system. Weight loss is related to reduced risk of diabetes and CVD, decreased blood pressure, and improved lipid profile and blood glucose control (Pi-Sunyer, 1998).

Because excess abdominal fat is associated with higher mortality and increased risk of chronic disease, including diabetes and heart disease, it makes sense to look at components in the diet that can potentially be associated with lower central adiposity. The NHLBI clinical guidelines for overweight and obesity recommend focusing treatment on altering dietary factors to prevent development of obesity and allow for moderate weight loss (Pi-Sunyer et al.,

1998) and encouraging non-fried fish intake might be a valuable intervention towards these goals. While the beneficial effects of fish intake on central adiposity may be modest, weight loss of 7% to 10% over a period of six months to one year is recommended to reduce the underlying risk factors for metabolic syndrome (Grundy et al., 2005). Exploring methods to reduce central adiposity is important and current interventions are falling short.

CHAPTER THREE: METHODS

The purpose of the current prospective study is to investigate the longitudinal relationships of average fish intake and repeated measures of waist circumference in a sample of 3,033 men and women. The data used in the current study were drawn from an on-going longitudinal, prospective study examining knee osteoarthritis called the Osteoarthritis Initiative (OAI) study. The primary goal of the OAI study is to alleviate or prevent the pain and disability associated with osteoarthritis by examining the progression and incidence of osteoarthritis in the knee (Nevitt, 2006). The present study examined data collected as part of the OAI study at enrollment and at 24 months and 48 months follow-up.

Research Hypotheses

When controlling for age, sex, race, presence of knee osteoarthritis, clinic assignment, smoking status, amount of fried fish eaten, energy intake, physical activity level, and level of depression, the amount of non-fried fish that a person consumes is negatively related to his or her waist circumference over time.

When controlling for age, sex, race, presence of knee osteoarthritis, clinic assignment, smoking status, amount of non-fried fish eaten, energy intake, physical activity level, and level of depression, the amount of fried fish that a person consumes is positively related to his or her waist circumference over time.

Study Population

The primary goal of the on-going OAI study is to alleviate or prevent the pain and disability associated with osteoarthritis by examining the progression and incidence of osteoarthritis in the knee (Nevitt, 2006) . To accomplish this goal, 4,674 men and women between the ages of 45 – 79 years who either currently had clinically significant knee osteoarthritis or were a high risk for developing knee osteoarthritis were recruited between February 2004 and May 2006 to participate in the study. Additionally, 122 people were recruited with the same demographic characteristics but no risk factors for or presence of knee osteoarthritis. In total, 4,796 people enrolled in the OAI study.

Potential participants were initially contacted through the mail and invited to contact a local OAI clinic if interested in the study. Enrollment into the OAI study was open to men and women between the ages of 45 and 79 years of any ethnic group. Major exclusion criteria for the OAI study were inflammatory arthritis (Rheumatoid Arthritis), bilateral end-stage knee osteoarthritis, and contraindications to use of 3-Tesla magnetic resonance imaging (MRI) (i.e. higher power) systems. An initial eligibility interview was conducted over the phone with each interested person and eligible participants were invited to complete an on-site screening visit. Participants who completed an on-site screening visit where then invited to complete the enrollment clinic visit. Prior to the on-site enrollment visit, participants were given self-administered questionnaires to complete and return, including the Block Brief 2000 Food

Frequency Questionnaire (Block, Hartman, & Naughton, 1990) and the Center for Epidemiological Studies Depression Scale (CES-D) for depressive symptoms (Radloff, 1977). At the enrollment visit, self-administered questionnaires were reviewed and collected and the Physical Activity Scale for the Elderly (PASE) score was assessed. Baseline waist circumference was measured at the enrollment visit.

A total of 4,796 men and women aged 45-79 completed the baseline measures in the OAI study. Prior to the enrollment visit, participants were assigned to one of three cohorts: (1) Symptomatic (progression) cohort, including those symptomatic for knee osteoarthritis, (2) Risk factors (incidence) cohort, including those non-symptomatic but with the presence of eligibility risk factors for knee osteoarthritis, and (3) non-exposed cohort, including people with no symptoms or eligibility risk factors for knee osteoarthritis.

The OAI study is publicly funded by the National Institutes of Health (NIH) and privately funded by multiple pharmaceutical companies (Nevitt, 2006). Data were collected from the following clinical centers in the United States

- Ohio State University
- University of Maryland School of Medicine and John Hopkins University School of Medicine
- University of Pittsburgh School of Medicine
- Brown University School of Medicine and Memorial Hospital of Rhode Island

University of California, San Francisco School of Medicine served as the data coordinating center.

Informed consent according to federal guidelines was obtained at each collection site prior to any participation in the OAI. Example consent forms were provided to each collection site, but each site was able to modify forms according to their local IRB requirements (Nevitt, 2006) .

Study Design

The current study was a longitudinal, prospective analysis that examined the relationships between baseline levels of average non-fried and fried fish intake and repeated measures of waist circumference. The current study utilized three waves of the OAI study data: baseline, 24 months follow-up and 48 months follow-up. Of the 4,796 people who enrolled in the OAI study, 1,763 of those participants were not included in the final study analysis due to missing data. 3,033 participants were included in the study analysis group.

The dependent variable was waist circumference (cm), represented by repeated measures of waist circumference taken at baseline, 24 months and 48 months. The major predictor variables are the average amount of non-fried fish eaten (cups/week) and the average amount of fried fish eaten (cups/week), collected from a food frequency questionnaire taken at baseline. Associations will be adjusted for age, race, sex, energy intake, smoking status, presence of knee osteoarthritis, clinic assignment, level of physical activity, level of depression, and time between visits.

The longitudinal design of this study distinguishes it from many of the cross-sectional studies that have been performed on fish and fish oil intake and anthropometric measures of body composition and associated health risks (Lai et al., 2013). This is the first study to examine the effect of fish intake on waist circumference using generalized estimating equations (GEE).

The Institutional Review Board of Middle Tennessee State University approved the research protocol to conduct this study [Appendix A].

Measurements

At enrollment, which took place between 2004 and 2006, anthropometric measurements including height, weight and waist circumference were taken and a self-administered food frequency questionnaire was completed. Other lifestyle characteristics, including level of physical activity and level of depression, were assessed. Follow-up anthropometric measurements and level of physical activity score were taken at approximately 24 months and 48 months.

Waist circumference.

The primary outcome of interest in this study was waist circumference (cm), measured at baseline, 24 months and 48 months follow-up. Waist circumference is a time dependent variable. Waist circumference has been found to be positively correlated with level of visceral abdominal fat and is considered a clinically relevant measure of central adiposity (Barreira et al., 2012). Central adiposity is typically defined as having a waist circumference over 102 cm (40 in) in men and over 88 cm (35 in) in women (Pi-Sunyer et al., 1998).

Waist circumference can be measured in several ways and it is important to consider measurement protocol when examining waist circumference. The World Health Organization protocol calls for measurement of waist circumference halfway between the "lower margin of the last palpable rib and the top of the iliac crest" (World Health Organization, 2011).

Many studies that measure waist circumference measure at the level of the umbilicus and natural waist line, but there is evidence that measuring waist circumference at the maximum circumference may give a better indicator of amount of adipose tissue. In the OAI study, waist circumference was measured at the point of maximum circumference, with a flexible, inelastic fiberglass tape over bare skin while the participant was standing (Nevitt, 2006). Measurements were recorded to the nearest 0.1 centimeters (cm) and was included as a continuous variable in the current study.

Weight and height were also measured at baseline, 24 months and 48 months. Weight was measured twice for each participant with a standard balance beam scale and recorded in kilograms (kg). Participants wore light clothing and no shoes or heavy accessories when being weighed. Height was measured using a wall-mounted stadiometer and recorded in millimeters (mm). Height measurements were taken without shoes and each participant was measured twice (Nevitt, 2006).

Fish intake.

The primary exposures of interest in this study were the amount of non-fried fish and the amount of fried fish eaten, measured in cups per week. At baseline, OAI participants were given a validated, self-administered, semi-quantitative food frequency questionnaire (FFQ), the Brief Block Questionnaire 2000 (Block et al., 1990). There were 2 questions about non-fried fish intake and 2 questions about fried fish intake, all with categorical responses. For questions 2 and 4, a picture of portion sizes was provided.

1. How often in the past 12 months did you eat fried fish or fish sandwich, at home or in a restaurant?
    - Nine response options: 1 = never, 2 = a few times per year, 3 = once per month, 4 = 2-3 times per month, 5 = once per week, 6 = twice per week, 7 = 3-4 times per week, 8 = 5-6 times per week, and 9 = everyday
2. How much fried fish did you eat each time?
    - Four response options: 1 = ¼ cup, 2 = ½ cup, 3 = ¾ cup and 4 = 1 cup
3. How often in the past 12 months did you eat any other fish or shellfish, not fried, including tuna?
    - Nine response options: 1 = never, 2 = a few times per year, 3 = once per month, 4 = 2-3 times per month, 5 = once per week, 6 = twice per week, 7 = 3-4 times per week, 8 = 5-6 times per week, and 9 = everyday
4. How much non-fried fish did you eat each time?
    - Four response options: 1 = ¼ cup, 2 = ½ cup, 3 = ¾ cup and 4 = 1 cup

To examine fish intake, a multiplicative, continuous index score was created for the amount of non-fried fish eaten and the amount of fried fish eaten weekly. The original categorical responses were converted into continuous responses based on how many times fish was eaten on a weekly basis. The score for how many times fish was eaten weekly was multiplied by how much fish was eaten each time (in cups) to create a multiplicative index for the amount of non-fried fish eaten weekly and the amount of fried fish eaten weekly (cups/week).

There is evidence that frying fish destroys up to 70% of the EPA and 85% of the DHA that was originally found in the raw fish (Stephen et al., 2010), suggesting that non-fried fish and fried fish should be examined separately. Self-administered FFQs have been found to be valid to estimate long-term fish and seafood intake when compared to adipose tissue levels of LC n-3 PUFA (Wallin et al., 2014) but FFQs were found to overestimate fish intake compared to daily food records (Birgisdottir, Kiely, Martinez, & Thorsdottir, 2008). It is acceptable to use fish intake at baseline in this repeated measures design because people tend to eat the same diet over time (Bernstein et al., 2016).

Covariates and factors.

In addition to the primary predictors of non-fried and fried fish intake, other variables were examined in the current study analysis. Covariates included as continuous variables in the model with fish intake were age at baseline (years), average energy intake at baseline (calories per day), time between visits (years), depression score at baseline (CES-D score) and repeated measures of physical activity (PASE). Factors included as categorical variables in the model were sex, race, and presence of symptomatic knee osteoarthritis. Height has been found to be uncorrelated to adiposity (Barreira et al., 2012) and was not included in the final analysis.

The analysis utilized in the current study assumes an absences of high multicollinearity among the predictor variables and there was significant collinearity in the model when weight was included, therefore weight was taken out of the final analysis. Waist circumference has an independent effect on health risks and disease prevalence (Katzmarzyk et al., 2013), but weight and waist circumference are both measures of physical presence and both commonly used to assess overweight and obesity.

Time between visits.

Because the current study used a transitional model to examine change in waist circumference over time, a new variable was created to represent how much time had passed between repeated measurements. The number of days between the date of the previous measurement of waist circumference and the date of the repeated measurement was counted and then divided by 365.25 to calculate time between visits in years. Time was included in the final model as a continuous variable. Waist circumference tends to increase over time (Jackson et al., 2015; Ladabaum et al., 2014).

Sex.

Sex was recorded as man = 0 or woman = 1. An interaction of gender and waist circumference in predicting level of visceral fat has been found, with higher levels of visceral fat found in men compared to women at the same BMI level (Mongraw-Chaffin et al., 2015).

Age.

Age in years at baseline was included as a continuous variable.

Race.

Information on race was collected at baseline. In the current study, race was coded as 0 = white, 1 = black and 2 = Hispanic or other race.

Energy intake.

Energy intake was estimated from the baseline FFQ and recorded as average daily calorie intake (kcal).

Physical activity.

Level of general physical activity was measured using the Physical Activity Scale for the Elderly (PASE) (Washburn, Smith, Jette, & Janney, 1993). The PASE asks about level of activity in the past 7 days around three domains: leisure activities, household activities and occupational activities. The PASE score is a weight summation of 12 items that ranges from 0 to 400. The PASE has been found to be a valid tool to use when accessing older adults with pain for level of physical activity (Dinger, Oman, Taylor, Vesely, & Able, 2004; Harada, Chiu, King, & Stewart, 2001; Martin et al., 1999). PASE was collected at baseline, 24 months and 48 months. Waist circumference is negatively associated with level of physical activity (Ladabaum et al., 2014).

Depression.

Level of depression was recorded at baseline as a CES-D score. The CES-D 20-item questionnaire that has is a continuous score that ranges from 0 to 60, with higher scores indicating higher levels of depressive symptoms (Radloff, 1977)

Presence of knee osteoarthritis.

Presence of knee osteoarthritis was included as a dichotomous categorical variable (yes/no). Participants who were symptomatic for knee osteoarthritis at baseline where put in the symptomatic cohort and were coded yes for presence of symptomatic knee osteoarthritis. Participants who were at risk for knee osteoarthritis but without symptoms of knee osteoarthritis and control participants were combined and coded no for presence of knee osteoarthritis

Smoking.

Current smoking status was included in the model as a dichotomous categorical variable (yes/no).

Centering of variables.

To facilitate interpretation of the parameter estimates, the mean of each continuous independent variable was subtracted from its values so that the variables were centralized around the mean of zero. Failure to centralize variables can cause more model fitting failure with GEE analyses compared to traditional regression analyses (Burton, Gurrin, & Sly, 1998).

Study Analysis

Data for the current analyses were obtained from the OAI study website, merged as needed and converted for use in IBM Statistical Package for Social Science (SPSS) for Windows, Version 23.0. All statistical analyses were performed using SPSS. The study analysis was restricted to participants with complete information on fish intake and other covariates, and waist circumference at all time points (n = 3033). Of the original 4,796 OAI participants, 36.8% (n=1763) were excluded due to incomplete data.

Generalized estimating equations (GEE).

Generalized estimating equation (GEE) models were applied to examine the relationship between non-fried and fried fish intake at baseline (cups/week) and waist circumference (cm) at baseline, 24 months and 48 months. GEE is an extension of generalized linear models (GLM) that supports correlated data, such as repeated measurements. Because there is within-subject correlation expected between measurements of waist circumference across time (errors in prediction at 24 months are correlated with errors in prediction at 48 months), traditional repeated measures linear regression would produce regression estimates that are less efficient than they would be if this within-subject correlation is incorporated in the analysis (Ballinger, 2004; Zeger & Liang, 1986).

GEE uses quasi-likelihood (QL) to estimate regression coefficients through a repetitive series of linear regression models with each repeated measure (Arnau, Bono, Bendayan, & Blanca, 2016). These coefficients are then corrected for the correlation among the repeated measurements and used to create final coefficients for the predictor variables that represent the relationship with the dependent variable across all time points (Twisk, 2003). The final regression coefficient represents a population average of the between-subject effects and within-subject effects. For every one-unit increase in a variable across the population studied, GEE b coefficients represent how much the average waist circumference over time would change (Liang & Zeger, 1986; Zeger & Liang, 1986).

Model specification.

There are a number of model specifications that need to be described when analyzing GEE models. The nature of the working correlation matrix for repeated measures must be specified. There are multiple correlation structures to choose from with GEE and the simplest correlation structure that fits the data well is typically the best choice (Twisk, 2003). In a GEE analysis with a large sample size, parameter estimates are efficient and consistent against incorrect specification of the correlation matrix structure (Arnau et al., 2016; Zeger & Liang, 1986). In the current study, a first-order autoregressive correlation structure was selected to represent the correlation found in the repeated measurements of waist circumference. Longitudinal data is often examined with a first-order autoregressive correlation structure, which sets the within-subject correlations as an exponential function of the lag period between repeated measurements (Ballinger, 2004). A variance-covariance estimator must also be specified in GEE. The choice of estimator is either robust or model-based, and the robust estimator is typically recommended because consistent parameter estimates are produced even when the correlation matrix structure is misidentified (Hardin & Hilbe, 2007)

The distribution of the dependent variable must be specified because GEE does not assume normal distribution. In the current study, the distribution of waist circumference across time was found to be positively skewed (0.236) so waist circumference was modeled with a gamma distribution. The gamma distribution is appropriate for positively skewed, continuous dependent variables when there are no negative values (Garson, 2013). A link function must also be specified in GEE analysis. The link function selected can transform a dependent variable so that it is linearly related to the predictor variables. In the current study, linearity between the predictors and waist circumference was assumed and the identity link function was selected. With the identity link function specified, there is no transformation of the dependent variable (Garson, 2013). The combination of gamma distribution and identity link function are commonly used to model duration data.

As part of the model specification in GEE, a subject identification variable must be selected as well as a within-subjects variable that indicates the repeated measurement condition. In the current study, the subject identification variable was individual participant identification numbers. A new categorical variable was created to identify the measurement wave of the repeated measurement (months = 0, 24, or 48). The *months* variable was used as the within-subjects variable to identify repeated measurements for analysis.

For model building, a series of adjusted GEE models were fitted and compared. The models of best fit were assessed using Corrected Quasi Likelihood under Independence Model Criterion (QICC) (Pan, 2001). QICC is a generalization of Akaike's information criterion (AIC), a goodness-of-fit statistic commonly used for likelihood-based model selection. When comparing two or more models, the model with the lowest QICC value is typically preferred, though the research hypothesis should also guide which effects are included in the model (Hardin & Hilbe, 2007). All 2-way interactions between the independent variables were assessed to determine the best set of predictor variables in the final model. Results of the analysis include estimated regression coefficients for the significant effects (*B*) and standard errors (*SE B*), estimated marginal means (adjusted means) and estimates of significance. Estimated marginal means are the mean waist circumferences in the groups created by the factor variables, when holding all other covariates in the model constant. Significant variables were identified by $p < 0.05$.

The GEE analysis was performed with repeated waist circumference measurements as the dependent variable. The basic model was adjusted for age (years), sex (male/female), race (white/black, Hispanic), time between visits (years), presence of symptomatic knee osteoarthritis (yes/no), clinic assignment, depression score (CES-D score), current smoking status (yes/no), level of physical activity (PASE score), and energy intake (kcal/day). Level of physical activity was modeled as a time-varying covariate, all other covariates were based on baseline measurements.

Assumptions.

As will all statistical procedures, GEE analysis make several assumptions. Observations are assumed to be dependent within subjects and independent between subjects. The correlation structure that represents the within-subject dependencies is estimated as part of the model. Predictor variables are assumed to be in a linear relationship with the link function of the dependent variable specified in the model. The current study utilized an identity link function, meaning there was no transformation of the dependent variable. Therefore, this assumption was not tested, however the residual plot from GEE analysis did not indicate a lack of linearity.

GEE assumes an absences of high multicollinearity among the predictor variables. There was significant collinearity in the model when weight was included, therefore weight was taken out of the final analysis. Waist circumference has an independent effect on health risks and disease prevalence (Katzmarzyk et al., 2013), but weight and waist circumference are both measures of physical presence and both commonly used to assess overweight and obesity.

GEE does not assume normal distribution for the dependent or independent variables. Independent variables may be of any distribution that maintains the linearity of the link function and the dependent variable may be from any of the exponential family of distributions, including normal and gamma (Garson, 2013).

Residuals analysis.

Residuals are the difference between the observed values for waist circumference and those predicted by the GEE model. A scatterplot of residuals versus the predicted values of waist circumference was used to observe the assumption that residuals are randomly distributed (Hardin & Hilbe, 2007). A histogram of residuals was inspected and a Kolmogorov-Smirnov (K-S) test was used to test for normality of residuals. When using an identity link model in GEE, homogeneity of variance for the range of the dependent variable is assumed (Garson, 2013). Homoscedacity was tested using the Levene's test. For other link function selections in GEE, there is no assumption of homogeneity of variance.

CHAPTER FOUR: RESULTS

The primary purpose of the current study was to investigate the longitudinal relationship between baseline measurement fish intake and repeated measures of waist circumference. Generalized estimating equations (GEE) was used to estimate the relationship between weekly fish intake (cup/week) on waist circumference (cm). The GEE analysis included additional predictor variables: age (years, continuous), race (white, black, Hispanic), sex (male/female), presence of symptomatic knee osteoarthritis (yes/no), clinic assignment, current cigarette smoking status (yes/no), energy intake (kcals/day, continuous), level of physical activity (PASE score, continuous), and level of depression (CES-D score, continuous).

Sample Demographics

There were 4,796 participants who completed the enrollment process for the OAI study. GEE analysis is capable of including missing data, but with repeated measures, if it is likely that the probability of missing data depends on the previous value of the outcome variable, parameter estimates may be less efficient (Zorn, 2001). For this reason, 1,743 participants with missing data were excluded from the current study. Data from 3,033 participants were used in the study analysis.

Table 1 shows baseline characteristics for participants included in the study analysis. The majority of the participants were white (83.20%) women (56.84%) with an average age of 61.41 years old at baseline. Table 2 displays descriptive information for repeated measures waist circumference and PASE score for participants included in the study analysis. The average waist circumference of participants at baseline (*M* = 102.34 cm) would be classified as an elevated risk factor in both men and women. Waist circumference increased over time in the current sample.

Table 3 shows baseline demographic information for those excluded from the analysis sample because of missing data.

Model Selection

GEE was used to test transition models that describe the relationships between fish intake at baseline and waist circumference across time. The QICC value was compared between analyzed models and was used to guide final model selection. All 2-way interactions were tested and compared to a main effects model. The interaction effects model QICC value was 381.293 compared to a QICC value of 160.084 for the main effects model, therefore the main effects model was selected for the final model. The best fitting GEE model to predict waist circumference across time included the following significant predictors: non-fried fish intake, fried fish intake, age, sex, race, presence of knee osteoarthritis, clinic assignment energy intake, level of physical activity, level of depression, and time between visits. The working correlation matrix for the final model is summarized in Table 4. Regression parameter estimates and standard errors for the final model are shown in Table 5.

Table 1

Baseline Characteristics of Participants Included in Study Analysis (N=3,033)

Characteristic	***M***	***SD***
Waist circumference (cm)	102.34	12.70
Age (years)	61.41	9.06
Non-fried fish intake (cups per week)	0.66	0.83
Fried fish intake (cups per week)	0.24	0.43
Energy intake (kilocalories per day)	1,398.09	578.05
Physical activity score (PASE)	163.64	80.70
Depression score (CES-D)	6.11	6.50
	n	***%***
Sex		
Men	1,294	43.16
Women	1,739	56.84
Race		
White	2,531	83.30
Black	432	14.15
Hispanic and other	70	2.55
Current smoker		
Non-smoker	2,869	94.76
Smoker	164	5.24
Symptomatic knee osteoarthritis		
Yes	845	27.84
No	2,188	72.16
Site of treatment		
Clinic 1	459	15.11
Clinic 2	693	23.02
Clinic 3	919	30.35
Clinic 4	755	24.65
Clinic 5	207	6.87

Table 2

Characteristics of Repeated Measurements of Participants Included in Study Analysis (N=3,033)

	Measurement Time					
	Baseline		24 months		48 months	
	M	*SD*	*M*	*SD*	*M*	*SD*
Waist circumference (cm)	102.34	12.70	103.27	12.57	103.48	12.66
Physical activity score (PASE)	163.64	80.70	154.24	79.72	154.34	81.33

Table 3

Baseline Characteristics of Participants Not Included in Study Analysis Due to Missing Data (N = 1,763)

Characteristic	*M*	*SD*
Waist circumference (cm)	102.60	13.18
Age (years)	60.73	9.39
Non-fried fish intake (cups per week)	0.62	0.81
Fried fish intake (cups per week)	0.28	0.59
Energy intake (calories per day)	1,405.34	692.39
Physical activity score (PASE)	155.94	85.31
Depression score (CES-D)	7.51	7.70
	n missing	*% missing*
Sex		
Men	698	35.04
Women	1,065	37.98
Race		
White	1,239	32.86
Black	434	50.12
Hispanic and other	86	55.10
Missing	4	100.00
Current Smoker		
Yes	149	47.60
No	1,552	35.11
Missing	62	100.00
Symptomatic knee osteoarthritis		
Progression	545	39.21
Incidence	1,103	33.59
Not exposed controls	115	94.26
Site of treatment		
Clinic 1	286	39.21
Clinic 2	307	30.70
Clinic 3	387	29.60
Clinic 4	418	35.60
Clinic 5	355	63.17

Table 4

Working Correlation Matrix for Waist Circumference

Time of Measurement	Month 1	Month 2	Month 3
Month 1	--	0.796	0.633
Month 24	0.796	--	0.796
Month 48	0.633	0.796	--

A priori assumption: First-order autoregressive structure

Model: (Intercept), OA Cohort, Clinic Site, Sex, Race Ethnicity, Age, Calories per day, Non fried fish consumption, fried fish consumption, Physical activity, Depression, Time between visits

Table 5

Summary of Generalized Estimating Equations Analysis for Variables Predicting Waist Circumference Across Time (N = 3,053)

Variable	*B*	*SE B*	*p*
Main Effects:			
Intercept	103.554	0.747	< .001
Non-fried fish intake (cups per week)	-0.677	0.288	.019
Fried fish intake (cups per week)	1.686	0.575	.003
Age (years)	0.115	0.024	< .001
Time between visits (years)	0.277	0.037	< .001
Energy intake (kilocalories per day)	0.002	0.001	< .001
Level of physical activity (PASE)	-0.006	0.001	< .001
Depression score (CES-D)	0.082	0.037	.025
Sex			
Women	-1.43	0.45	.001
Men	(reference)		
Race			
Black	2.59	0.72	< .001
Hispanic or other	-6.55	1.31	< .001
White	(reference)		
Symptomatic knee osteoarthritis			
Yes	3.70	0.48	< .001
No	(reference)		
Site of treatment			
Clinic 5	0.60	1.10	.586
Clinic 4	1.70	0.71	.017
Clinic 3	3.22	0.74	< .001
Clinic 2	5.30	0.77	< .001
Clinic 1	(reference)		

Note. Corrected Quasi Likelihood under Independence Model Criterion (QICC) = 160.084

The model intercept was significant (B = 103.55, $p < .001$), meaning that among the study participants, a white man who is non-symptomatic for knee osteoarthritis and has a mean value on the continuous predictors had a waist circumference of 103.55 cm.

Fish Intake

Non-fried fish intake (cups/week) was negatively associated with waist circumference across time (B = -0.68, $p < .001$). For every additional cup of non-fried fish eaten each week, waist circumference decreased an average of 0.68 cm. These results were adjusted for age, sex, race, presence of knee osteoarthritis, clinic assignment, energy intake, fried fish intake, level of physical activity, level of depression and time between visits.

Fried fish intake (cups/week) was positively associated with waist circumference across time (B = 1.69, p = .003). For every additional cup of fried fish eaten each week, the average waist circumference measurement increased an average of 1.69 cm. These results were adjusted for age, sex, race, presence of knee osteoarthritis, clinic assignment, energy intake, non-fried fish intake, level of physical activity, level of depression and time between visits.

Additional Predictors

All of the additional predictors examined in the final analysis, except for smoking status, were found to have significant associations with waist circumference across time. Regression parameter estimates and standard errors are displayed in Table 5. Observed and adjusted mean waist circumference for categorical predictors are found in Table 6.

Among the continuous predictor variables, when controlling for all other significant predictor variables, positive associations with waist circumference were found for age at baseline (*B* = 0.115, $p = .003$), energy intake (*B* = 0.002, $p < .001$), level of depression (*B* = 0.082, $p = .025$), and year since baseline (*B* = 0.277, $p < .001$). A negative association with waist circumference was found for level of physical activity (*B* = 0.277, $p < .001$).

Among the categorical predictor variables, when controlling for all other significant predictor variables, women were found to have a significantly smaller waist circumference (*B* = -1.43, $p = .001$) compared to men. The adjusted means waist circumference for women was 101.11 cm compared to 102.54 cm in men. Race was also found to be a significant predictor of waist circumference. Compared to white people, black people had a significantly larger waist circumference (*B* = 2.59, $p < .001$, adjusted mean = 105.73 cm) and Hispanic people had a significantly lower waist circumference (*B* = -6.55, $p < .001$, adjusted mean = 96.60 cm).

Participants who were symptomatic for knee osteoarthritis had an average waist circumference 3.70 cm larger compared to those without symptomatic knee osteoarthritis ($p < .001$). Mean adjusted waist circumference among those symptomatic was 103.68 cm compared to 99.98 cm among those without symptomatic knee osteoarthritis.

Table 6

Summary of Estimated Marginal Means from Generalized Estimating Equations Analysis for Factor Variables Predicting Waist Circumference Across Time (N = 3,053)

Variable	Waist Circumference			
	Observed Mean	Adjusted Mean	SE	n
Sex				
Women	102.30	101.11	0.187	5,170
Men	103.99	102.54	0.187	3,857
Race				
Black	104.46	105.73	0.352	1,297
Hispanic or other	96.77	96.60	0.881	205
White	102.95	103.15	0.146	7,525
Symptomatic knee osteoarthritis				
Yes	106.00	103.68	0.251	2,519
No	101.87	99.98	0.156	6,508

Note. adjustments based on energy intake=1397.3275; non-fried fish intake=.6568; fried fish intake=.2444; depression score=6.12; PASE=157.45; time between visits=2.0018; age=61.38

Residual Analysis

A scatterplot of residuals versus the predicted values of waist circumference found the residuals to be appear to be randomly distributed, as shown in Figure 2. When using the identity link function in GEE, there is the assumption of normality and homoscedascity among the residuals. While a histogram of the residuals appears to be normally distributed, as illustrated in Figure 3, the results of a Kolmogorov-Smirnov (K-S) test find that the residuals are not normally distributed (p <.001). The Levene's test for homoscedascity was significant (p = .030) with the significant difference being between the 90cm-99cm group and the 100cm-109cm groups (p = .001).

The was no evidence of multicollinearity in the final model because coefficients remained relatively stable across changes to the model.

Violation of these assumptions should be noted, but by selecting the robust estimators of correlation as opposed to the model-based estimators, GEE can still predict parameter estimates (Hardin & Hilbe, 2007).

The results of the current analysis support both of the study hypotheses.

When controlling for age, sex, race, presence of knee osteoarthritis, clinic assignment, smoking status, amount of fried fish eaten, energy intake, physical activity level, and level of depression, the amount of non-fried fish that a person consumes is negatively related to his or her waist circumference over time.

When controlling for age, sex, race, presence of knee osteoarthritis, clinic assignment, smoking status, amount of non-fried fish eaten, energy intake, physical activity level, and level of depression, the amount of fried fish that a person consumes is positively related to his or her waist circumference over time.

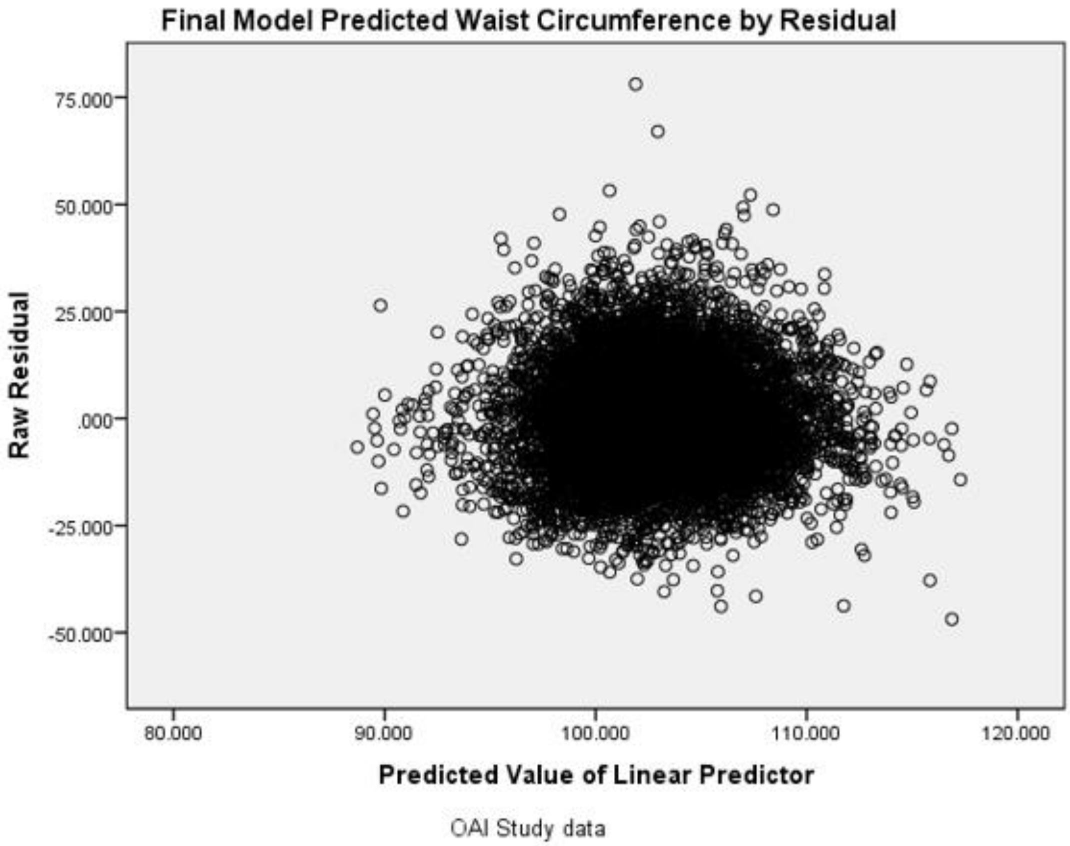

Figure 2. Residual plot from generalized estimating equations (GEE) analysis

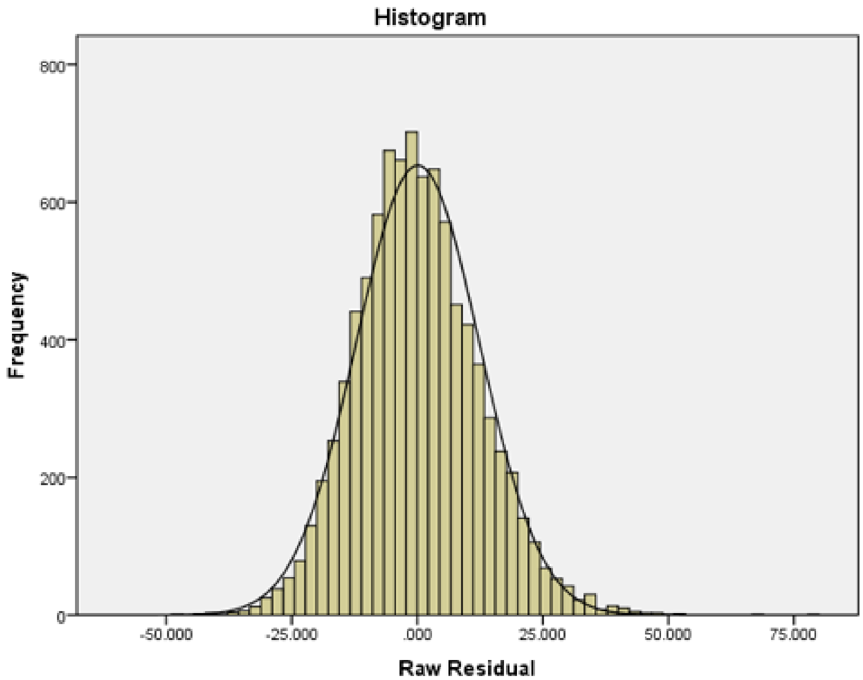

Figure 3. Histogram of residuals from generalized estimating equations (GEE) analysis

CHAPTER FIVE: DISCUSSION

The current study results found that non-fried fish intake was negatively related to waist circumference over time and fried fish intake was positively related to waist circumference over time among participants of the Osteoarthritis Initiative (OAI study). This relationship was independent of examined covariates, including energy intake and level of physical activity. This is the first known study to examine the relationship of non-fried fish and fried fish separately on waist circumference over time using a generalized estimating equations (GEE) analysis.

The American Heart Association (AHA), the Academy of Nutrition and Dietetics (AND) and the Dietary Guidelines for Americans 2015-2020 all recommend consuming approximately 8 ounce of fish per week to reduce risk of cardiovascular disease (Department of Health and Human Services & Agriculture, 2015; Lichtenstein et al., 2006; Vannice & Rasmussen, 2014). The average American falls short of that recommendation, eating only 4.27 ounces of fish each week (Papanikolaou et al., 2014). In the current study, average intake of non-fried fish was 0.66 cups per week and average intake of fried fish was 0.24 cups per week. While it is not possible to directly convert cups of food into ounces of food, 1 cup of flaked fish such as salmon or tuna can be estimated to be approximately 3.5 - 4 ounces of fish (Papanikolaou et al., 2014) so it is reasonable to suggest that 2 cups of fish is the equivalent of approximately 8 ounces of fish (Department of Health and Human Services & Agriculture, 2015).

The current study participants ate approximately 2.48 ounces of non-fried fish per week and approximately 0.90 ounces of fried fish each week. Even when combined for an approximate total fish intake of 3.38 ounces, the study participants ate a lower amount of fish than average Americans and did not meet the recommended 8 ounces of fish per week.

Considering these results, it may be that general population recommendations for fish intake should make a distinction between the non-fried and fried fish, specifically recommending non-fried fish intake and warning against fried fish intake. The American Heart Association (AHA) suggests that fish not be fried to avoid the added saturated and *trans* fats, but their recommendation does make a distinction between non-fried and fried fish (Lichtenstein et al., 2006).

The presence of knee osteoarthritis had a large positive effect on waist circumference compared to those without knee osteoarthritis. Those with knee osteoarthritis had an average waist circumference 3.70 cm larger than those without knee osteoarthritis. A different study examining OAI data found that elevated waist circumference was positively related to prevalence of knee osteoarthritis (Batsis et al., 2014). It maybe that those with symptomatic knee osteoarthritis are unable to exercise due to pain and loss of mobility. People with osteoarthritis and other chronic diseases may be at elevated risk for central adiposity, making them an important group to target with interventions aimed at improved body composition.

Central adiposity is a major health concern. Several studies have suggested that fish and seafood intake as well as LC n-3 PUFA intake may be a beneficial therapy against central adiposity and obesity in general. The results of the current study support this beneficial relationship, suggesting that it is a good idea to eat non-fried fish and to avoid eating fried fish, as well as exercise and avoid depression to maintain a healthy waist circumference.

There is a need to determine which lifestyle factors, include dietary choices, can decrease levels of central adiposity in the population. The current study supports the common recommendation to eat more fish, but suggests that emphasis should be place on eating more non-fried fish and eating less fried fish. Public health strategies should examine the role of fish intake in the fight against central adiposity.

Limitations

This study had limitations that should be discussed. While waist circumference is a clinically significant measure of central adiposity, computed tomography (CT)-measured visceral adipose tissue (VAT) is the best measure of true central adiposity. Waist circumference has been found to be highly related to level of VAT in the body (Barreira et al., 2012), but it does not specifically distinguish between excess adipose tissue accumulated in the abdominal cavity and the subcutaneous adipose tissue (SAT) found underneath the skin covering the abdominal region. It may be that there are other emerging anthropometric measurements that would be a better proxy measure of central adiposity, such as the supine sagittal abdominal diameter (SAD), which has been found to be a better predictor of cardiometabolic disorders than waist circumference or BMI (Kahn & Bullard, 2016).

Measurement of dietary intake of fish may also be an issue. Self-reported FFQ have been found to be a good measure of dietary intake, but it is possible fish intake records are not good. Also, dietary information was only collected at baseline. It is acceptable to use fish intake at baseline in this repeated measures design because it has people tend to eat the same diet over time (Bernstein et al., 2016), but it would be better to have repeated measures of dietary intake.

With longitudinal study designs, loss to follow up can be a major limitation. One advantage of the GEE statistical model is that all available data over the follow-up period can potentially be used, regardless of missing data (Hardin & Hilbe, 2007), but if it is likely that the probability of missing data depends on the previous value of the outcome variable, such as with repeated measurements the GEE parameter estimates may be less efficient (Zorn, 2001). For this reason, only participants with complete data for all study variables were included in the final analysis.

This prospective, longitudinal study of the relationship between fish intake and waist circumference found that non-fried fish is beneficially associated with waist circumference over time, and that fried fish has a detrimental relationship with waist circumference. This was not an experiment study, further study into the effects of non-fried and fried fish intake on waist circumference and other measures of body composition could help inform future dietary recommendations for fish and seafood intake.

Further research is needed to determine the different effects of fish protein compared to fish oil administered as a supplement. There is evidence that components of fish protein beyond EPA and DHA, including low saturated fat protein source and other vitamins and minerals, such as vitamin D and taurine, may be additionally beneficial for health.

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APPENDIX

APPENDIX A: IRB APPROVAL LETTER

IRB
INSTITUTIONAL REVIEW BOARD
Office of Research Compliance,
010A Sam Ingram Building,
2269 Middle Tennessee Blvd
Murfreesboro, TN 37129

MIDDLE TENNESSEE STATE UNIVERSITY

EXEMPT APPROVAL NOTICE

2/16/2016

Investigator(s): Tracy Morris & Norman Weatherby
Department: Health & Human Performance
Investigator(s) Email: tracy.morris@mtsu.edu
Protocol Title: "A longitudinal analysis of the effects of fish intake on central adiposity "
Protocol ID: 16-1163

Dear Investigator(s),

The MTSU Institutional Review Board, or a representative of the IRB, has reviewed the research proposal identified above and this study has been designated to be EXEMPT.. The exemption is pursuant to 45 CFR 46.101(b) **(4) Collection or Study of Existing Data**

The following changes to this protocol must be reported prior to implementation:

- Addition of new subject population or exclusion of currently approved demographics
- Addition/removal of investigators
- Addition of new procedures
- Other changes that may make this study to be no longer be considered exempt

The following changes do not have to be reported:

- Editorial/administrative revisions to the consent of other study documents
- Changes to the number of subjects from the original proposal

All research materials must be retained by the PI or the faculty advisor (if the PI is a student) for at least three (3) years after study completion. Subsequently, the researcher may destroy the data in a manner that maintains confidentiality and anonymity. IRB reserves the right to modify, change or cancel the terms of this letter without prior notice. Be advised that IRB also reserves the right to inspect or audit your records if needed.

Sincerely,

Institutional Review Board
Middle Tennessee State University

NOTE: All necessary forms can be obtained from www.mtsu.edu/irb.