Relationships Between the Features of Metabolic Syndrome and Fatty Acids in the Diet, Plasma, and Adipose Tissue of Healthy Older Adults

THESIS

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Abstract

Introduction

The metabolic syndrome (MetS) is a cluster of disturbances including central adiposity, dyslipidemia, hypertension, and hyperglycemia. The prevalence of MetS is increasing and is estimated to affect 34% of the adult population, including nearly 50% of males and 57% of females over age 60. The risk of MetS is potentially modifiable with changes in the dietary fatty acid composition. This study seeks to determine the relationships between dietary, plasma, and adipose fatty acid and the components of MetS.

Methods

We conducted a cross-sectional study of 142 healthy older adults from Ohio aged 60-88, examining fat quality as measured by 7-day weighed food record (FR), diet history questionnaire (DHQ), plasma fatty acid composition, and adipose fatty acid composition. We then evaluated how these measurements relate to various components of MetS.

Results

Dietary saturated fat, monounsaturated fat, and cholesterol, as reported in the DHQ, predicted MetS diagnosis. Dietary monounsaturated fat as reported on the DHQ and n-3 fatty acids ALA, EPA, and DHA, as reported on the FR, were directly related to the
number of features of MetS. Increased levels of plasma 16:0, 16:3n4, 18:1n9, and 20:1n9 were predictive of MetS. Plasma saturated and monounsaturated fatty acids were positively correlated, while plasma polyunsaturated fatty acids were negatively correlated, with triglyceride levels. Plasma ratios of 16:1 to 16:0 and 18:1 to 18:0 were positively correlated with triglyceride levels. The ratio of plasma 18:1 to 18:0 was also negatively correlated to HDL levels. Adipose 16:1n7 and 18:4n3 were decreased in subjects with increasing numbers of MetS features. Adipose 16:0 was correlated with each feature of MetS.

Conclusions

Dietary, plasma, and adipose fatty acid composition were shown to have relationships to components of metabolic syndrome in healthy older adults. Specifically, we have shown a relationship between n-3 fatty acid consumption and the presence of MetS components, suggesting a potential role for increased n-3 consumption and the reduction of MetS prevalence.
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Chapter 1: Introduction

The metabolic syndrome (MetS) is a cluster of metabolic disturbances that include central obesity, dyslipidemia, hypertension, and hyperglycemia. In the 1988-1994 NHANES III survey, MetS was estimated to affect 29% of the adult US population\(^1\). Since then, the prevalence of MetS has increased and is estimated to affect 34% of the adult population, including nearly 50% of males and 57% of females over age 60\(^2\).

Because MetS is associated with an elevated risk of both type 2 diabetes and cardiovascular disease, an increased incidence of MetS predicts for increased morbidity and mortality attributed to these diseases\(^3\). A meta-analysis of 21 studies found that the presence of MetS increases the risk of mortality from all causes by 35%, including a 74% increase in the relative risk of cardiovascular disease and a 76% increase in the relative risk of stroke\(^4\). Similarly, normal weight individuals with MetS have four times the risk of developing type 2 diabetes as those without the syndrome, while obese individuals with MetS have 10 times the risk of incident diabetes compared to unaffected, normal weight controls\(^5\). The increased risk of death from MetS also holds true specifically for older adults. A study of an Italian cohort of adults over 65, found an increased risk of all cause mortality (hazard ratio 1.41) and cardiovascular mortality (hazard ratio 1.60) in subjects with MetS\(^6\).
There is no internationally agreed upon definition of MetS. However, in the United States, one common definition is derived from the Adult Treatment Panel III where MetS is defined as having three or more of the following criteria:

1. Waist circumference greater than 103 cm in males or 88 cm in females.
2. HDL less than 40 mg/dL in males or 50 mg/dL in females or drug treatment for low HDL.
3. Triglycerides greater than 150 mg/dL or drug treatment for high triglycerides.
4. Fasting blood glucose greater than 100 mg/dL or drug treatment for high fasting glucose.
5. Blood pressure greater than 130/85 mmHg or drug treatment for high blood pressure.
Chapter 2: Literature Review

The common etiology of the MetS cluster may be inflammation due to central obesity and the resulting insulin resistance\textsuperscript{8}.

2.1 Insulin action

Insulin, produced by the pancreas in response to elevated blood glucose levels, binds to its receptor on the surface of a target cell. The receptor autophosphorylates on tyrosine residues and then phosphorylates insulin receptor substrates (IRS), also on tyrosine residues. IRS then activates multiple pathways. In the PI3K pathway, active IRS binds the SH2 domain of PI3K, activating it so that it can phosphorylate and activate PI3-dependent kinases (PDK1, PDK2). PDK activates atypical PKC and Akt (also known as PKB). Akt then activates AS160 which, with required participation of PKC, stimulates the translocation of Glut4 to the cell surface to allow glucose into the target cell\textsuperscript{9}.

In the liver, Akt also phosphorylates Foxo1, which is then inactive because it is excluded from the nucleus. This prevents the transcription of gluconeogenic enzymes like glucose-6-phosphatase and phosphoenolpyruvate carboxykinase (PCK1), avoiding the unnecessary de novo production of glucose in the liver when blood glucose levels are adequate\textsuperscript{10}. 

3
In insulin resistance, IRS is phosphorylated, not on activating tyrosine residues, but on inhibiting serine residues, which blocks signal transduction. This is a normal negative feedback process in insulin signaling, but it can also aberrantly occur by inflammatory mediators such as SOCS and nitric oxide, providing a link between inflammation and insulin resistance\(^{11}\).

A second potential mechanism of insulin resistance is an increase in the activity of phosphatases that remove the activating phosphorylation from the insulin receptor. In obese rodents, there is increased expression of the protein tyrosine phosphatase 1B (PTP1B) that acts on the insulin receptor. Similarly, in human subjects a significant correlation has been found between the activity of these phosphatases and BMI\(^9\).

Insulin encourages the growth of fat stores by promoting preadipocyte differentiation, and activating glucose transport and lipogenesis. Adipocytes are highly responsive to the lipogenic actions of insulin, potentially via the activation and overexpression of SREBP-1c. Therefore, increased fat accumulation can occur despite a state of systemic insulin resistance\(^{12,13}\).

### 2.2 Obesity and inflammation

Increased abdominal obesity has been proposed to lead to higher levels of inflammatory molecules because visceral adipose produces higher levels of pro-inflammatory adipokines such as IL-6 when compared to subcutaneous adipose\(^8,14\). Similarly, weight loss on a very low calorie diet over 28 days reduces the expression of these inflammatory
genes in the adipose tissue of obese subjects while increasing the expression of anti-inflammatory genes such as IL-10. However, expression is not reduced to the level of non-obese control subjects. This observation offers further support for the idea that obesity results in a chronic state of inflammation. However, increased inflammation is not merely due to an increase in adipose tissue mass. A recent case-control study of adults with and without MetS found that differences in adipokine levels from different depots persist even after adjusting for adiposity, suggesting the location of the adipose may be more important than the amount of tissue. This inflammation may then contribute to insulin resistance and other features of MetS.

Therefore, it appears that there is something metabolically different about large adipocytes that make up visceral adipose tissue. When adipocytes isolated from subjects undergoing elective plastic surgery were fractionated by size, the fraction containing the largest adipocytes produced significantly more inflammatory adipokines like IL-6, IL-8, MCP-1 and leptin than the fraction of the smallest adipocytes. This was observed when the data was analyzed as adipokine release per cell or per unit cell surface area, suggesting cells are not producing more adipokine simply because they are larger, but possibly because of a unique feature of their metabolism. Expression of anti-inflammatory adipokines, however, was largely unchanged across the fractions.

In addition to the inflammatory milieu produced by the adipocytes, macrophages residing in the adipose tissue produce additional inflammatory cytokines. Macrophage concentration in visceral adipose has been found to increase with increasing BMI and
increasing adipocyte size so that increasing levels of overweight result in an increasingly inflammatory environment\textsuperscript{17} \textsuperscript{18}. A study of adipose tissue biopsies of 12 obese and 9 non-obese subjects undergoing elective surgery found that greater than 90\% of the crown-like clusters of macrophages were located around adipocytes that had undergone necrotic cell death\textsuperscript{19}. This can lead to a chronically inflamed environment as a large majority of adipose tissue TNF\alpha as well as iNOS and IL-6 may be produced by these infiltrating macrophages rather than the adipocytes themselves\textsuperscript{18}.

The recruitment of macrophages to the adipose tissue is believed to be a protective mechanism. As adipocytes grow in volume in obesity, they undergo stress leading to cell death, likely by necrosis rather than apoptosis. As a result, the plasma membrane ruptures, resulting in the release of lipid droplets that could be dangerous to the surrounding adipocytes as they provide an unregulated source of cholesterol and free fatty acids. It is hypothesized that macrophages are recruited to the adipose tissue to remove these lipid droplets\textsuperscript{19}. However, in removing the immediate threat, they introduce a chronic inflammatory state that is pathogenic in the long term. This inflammatory state produced by the adipocytes and macrophages may be further increased in insulin resistance because insulin normally suppresses proinflammatory transcription factors like NF-kB\textsuperscript{9}.

In contrast to the inflammation that results from expansion of the visceral adipose tissue, it is believed that subcutaneous adipose tissue is able to undergo a more healthy form of expansion. Unlike visceral tissue, subcutaneous fat has smaller fat cells and is well
vascularized to prevent necrosis and subsequent infiltration of macrophages that would result in inflammation\textsuperscript{20}.

Although obesity itself is associated with increased cardiovascular disease risk, because of the increased risk specific to abdominal obesity, waist circumference has been found to be a better predictor of risk than BMI\textsuperscript{21}. This increase in cardiovascular disease risk is clinically relevant. A meta-analysis of studies following over 250,000 subjects estimated that a 1 cm increase in waist circumference is associated with a 2\% increase in the risk of cardiovascular disease\textsuperscript{22}.

\subsection*{2.3 The interplay between the components of metabolic syndrome}

This increase in risk of cardiovascular disease is, in part, related to the effect of waist circumference on each of the features of MetS. Visceral adipocytes can act to increase insulin resistance in addition to their contribution to a chronic inflammatory state. Large abdominal adipocytes have been shown to have an increased rate of lipolysis, when compared to smaller peripheral adipocytes, resulting in excess non-esterified fatty acids (NEFA) in the liver which may increase de novo glucose production. This occurs even in the presence of insulin, which usually inhibits lipolysis\textsuperscript{23}. High NEFAs can also result in a reduction in glucose utilization to form fatty acids, further increasing hyperglycemia and, over the long term, resulting in chronically high insulin levels and eventual insulin resistance\textsuperscript{24}.
In addition to affecting glucose metabolism, high NEFAs can cause and/or result from increased hepatic triglyceride levels and VLDL secretion\textsuperscript{25}. VLDL secretion is further enhanced because insulin resistance impairs insulin's ability to cause the breakdown of apoB, which is an essential VLDL component\textsuperscript{26}. Together, central adiposity and insulin resistance may lead to the hyperlipidemia of MetS.

Abdominal obesity also affects HDL levels. Large abdominal adipocytes have been shown to bind and uptake HDL at an increased rate compared to smaller adipocytes. This increased clearance of HDL may be partly responsible for the decreased HDL levels in MetS\textsuperscript{27}.

Finally, a large waist-to-hip ratio has been found to increase the risk of hypertension. In a cohort of nearly 2000 American veterans, increases in waist-to-hip ratios were positively correlated with increases in blood pressure even after adjusting for age and BMI\textsuperscript{28}. Similarly, blood pressure levels have been found to be more closely correlated to central adiposity than peripheral adiposity as measured by subscapular and triceps skinfolds, respectively\textsuperscript{29}. A proposed mechanism for this effect is that the pro-inflammatory state in high visceral adiposity inappropriately activates the renin-angiotensin-aldosterone system and the sympatheic nervous system resulting in constricted blood vessels and higher blood pressure\textsuperscript{30}. 
2.4 Leptin

Although the most widely accepted hypothesis about the etiology of MetS is that it results from obesity and insulin resistance, alternative hypotheses have been proposed. One hypothesis is that MetS is a syndrome of leptin resistance or relatively low leptin levels. Genetic leptin deficiency or a leptin receptor mutation both produce the features of MetS while leptin treatment restores insulin sensitivity in these patients\textsuperscript{13}. A study of 147 obese subjects found that while leptin levels were correlated to both BMI and body composition, levels were increased only to in the presence of high subcutaneous, not visceral, fat. This suggests that those with visceral obesity produce less leptin compared to those with subcutaneous obesity\textsuperscript{31}. Leptin is believed to protect the body from high lipid levels via reducing lipogenesis and increasing fatty acid oxidation. A high level of visceral adiposity instead of peripheral adiposity may not produce sufficient leptin to protect the body, resulting in MetS\textsuperscript{13}.

Leptin's ability to increase fatty acid oxidation may also protect the body from ectopic fat deposits. Adipose tissue accumulation may be the body's method of safely storing excess calories in a tissue where fat will not cause lipotoxicity. Once the storage capacity of this tissue is met, lipids begin to accumulate in other tissues, and, as they are leptin deficient, leptin cannot act to prevent the buildup. The pathologies of MetS then develop. In this view, obesity is not a cause of MetS, but instead delays its onset\textsuperscript{20}. 

2.5 Aging

As previously mentioned, older adults have an increased prevalence of metabolic syndrome when compared to the general population. This may be due to several features of aging such as reduced hormone levels, decreased mitochondrial oxidation of fats, increased weight, and increased inflammation.

Aging is the breakdown of biological functions in an organism over time. In most species, including humans, females live longer than males. This phenomenon is likely due to differing hormone levels. Men experience a slow steady decline in testosterone levels throughout adulthood while women have a rapid drop in estrogen at menopause. In addition, sex hormone binding globulins increase with age, which further reduces the concentration of active hormone available. Together these mechanisms of reducing hormone availability may affect many of the common features of aging like abdominal obesity, weight gain, osteoporosis, decreasing muscle strength, urinary incontinence, and depression. They may also affect the risk of MetS, cardiovascular disease, and diabetes. For example, low insulin sensitivity and an increased risk of metabolic syndrome has been found to be associated with low testosterone levels in 40-80 year old men even after accounting for body mass, suggesting that testosterone may be protective against metabolic syndrome. In females, estrogen protects women against inflammation until menopause in part because estrogen may inhibit the NF-kB pathway and its resulting inflammatory action in the blood vessels. Estrogen also relaxes the blood vessels by
affecting the nitric oxide production pathway, lowering blood pressure\textsuperscript{32}. Both mechanisms might be protective against metabolic syndrome until later in life.

Another potential aging mechanism is a reduced oxidative capacity of the mitochondria. It is possible that accumulated damage to the mitochondrial DNA over time decreases the cell's ability to make energy, leading to cell death. This mechanism could also lead to a decreased ability of the cell to oxidize lipids, which would lead to ectopic lipid accumulation that contributes to decreased insulin sensitivity\textsuperscript{34}.

A final mechanism of aging and the increased prevalence of MetS is the level of inflammation. The inflammatory cytokine TNF-\(\alpha\) and, more controversially, IL-6 levels have been shown to increase in aging\textsuperscript{11}. In addition, increasing obesity causes a state of chronic inflammation that causes a loss of lean tissue, reduced immune function and reduced insulin sensitivity\textsuperscript{23}.

However, the increased prevalence of insulin resistance in older adults may be related to increased obesity in the group and not with aging itself. When elderly groups are matched for weight and activity level to younger controls, there is no significant difference in insulin sensitivity between the groups\textsuperscript{34}.

Body weight generally increases with age. In a study of 52 middle aged (average age of 50) and 122 young women (average age of 27), the middle-aged women had higher body fat mass, waist circumference, waist-to-hip ratio, and abdominal and visceral fat depots than the younger women\textsuperscript{35}.
The distribution of that weight remains a problem in older adults as it is in the general population. A Korean study using dual energy x-ray absorptiometry to assess visceral and subcutaneous adipose volume found that a male pattern of fat deposition (accumulating fat at the waist as opposed to the thigh) correlated with MetS risk factors in adults over 65 years of age, even after adjusting for age, gender, total body fat mass and other factors\(^36\). Conversely, as in the general population, femoral (thigh) fat may have a beneficial effect on health in older adults. The Health, Aging, and Body Composition (Health ABC) Study found that larger thigh fat depots are associated with healthier lipid levels in both men and women and improved glucose levels in men\(^37\).

2.6 Fatty acid composition and MetS

There is evidence that dietary fatty acid composition is related to MetS risk. A study of over 800 Tehranian adults found that those with the highest quartile of saturated fat intake had increased odds of having MetS\(^38\). Specifically, dietary 12:0, 14:0, and 16:0 have been found to raise cholesterol \(^39\). Conversely, substituting monounsaturated fat for saturated fat in a moderate fat diet has been shown to increase insulin sensitivity\(^40\).

N-3 fatty acid consumption has been associated with a decrease in the incidence of MetS. A prospective Korean study of over 3000 middle-aged men and women found a 67% decrease in MetS incidence in men who consumed fish daily compared to those who ate fish once per week. The intake of fatty fish was most strongly associated with triglyceride and HDL levels. No effect was seen in women\(^41\).
Plasma fatty acid composition may be representative of dietary fatty acid intake over several days prior to blood collection. In addition, it may also reflect endogenous fatty acid metabolism. Plasma fatty acid composition has been found to be related to the risk of MetS in several studies. In middle aged adults, those with MetS were found to have higher levels of plasma saturated and monounsaturated fats and lower levels of linoleic acid (18:2n6), arachidonic acid (20:4n6), and DHA (22:6n3). Consistent with this finding, plasma levels of 18:0 have been found to be increased in overweight subjects with MetS when compared to normal weight controls. In the same study, 16:1 was higher and 18:2n6 was lower in overweight subjects with MetS when compared to overweight or normal weight subjects without MetS. A separate study of Asian adults showed similar results: blood levels of total monounsaturated fatty acids, 16:1 and 18:1n9 were increased in subjects with increasing numbers of MetS risk factors. Investigating the individual components of MetS, plasma 16:1 content have been shown to be positively correlated to triglyceride levels and negatively correlated to HDL levels while 18:2n6 and 18:3n6 are negatively correlated with systolic and diastolic blood pressure.

Desaturases are enzymes that increase the number of double bonds in fatty acid hydrocarbon chains. As a marker of stearoyl-CoA desaturase activity, the desaturase index is calculated by finding the ratios of plasma 16:1/16:0 and 18:1/18:0 and may indicate high lipogenic activity. The desaturase index has been found to be higher in subjects with a greater number of metabolic syndrome risk factors and increased insulin resistance.
However, few studies have been undertaken to investigate the relationship between adipose fatty acid composition and MetS components. Adipose fatty acid composition is hypothesized to be a marker of habitual dietary fatty acid intake over the past 12 to 18 months. A study of Costa Rican adults found that subjects with higher adipose 20:4n6 fatty acid content had significantly higher odds of having MetS, even after the model was adjusted for multiple confounders. In contrast to findings in plasma that showed that high saturated fatty acid content was positively associated with MetS, the 14:0 and 18:0 content of subcutaneous abdominal adipose tissue were correlated with increasing insulin sensitivity.

2.7 Specific aims and hypothesis

The primary aim of our study was to investigate the relationship of components of the diet, the fatty acid composition of the plasma, and the fatty acid composition of the adipose tissue with features of MetS in healthy older adults. We hypothesized that the relationship between plasma fatty acids and the features of MetS would be similar in older adults as they are in the general population.
Chapter 3: Research

The following article is formatted for submission to the Journal of Nutrition, Health and Aging.

Relationships Between the Features of Metabolic Syndrome and Fatty Acids in the Diet, Plasma, and Adipose Tissue in Older Adults

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Abstract

Objectives

The metabolic syndrome (MetS) is a cluster of disturbances including central adiposity, dyslipidemia, hypertension, and hyperglycemia. The prevalence of MetS is increasing and is estimated to affect 34% of the adult population, including nearly 50% of males and 57% of females over age 60. This study sought to determine the relationships between dietary, plasma, and adipose fatty acid and the components of MetS.

Design

Cross-sectional study.

Setting

Urban and rural, non-institutionalized Ohio residents.

Participants

142 healthy older adults aged 60-88.

Measurements

Fat quality as measured by 7-day weighed food record, diet history questionnaire (DHQ), plasma fatty acid composition, and adipose fatty acid composition. Dependent variable measurements were the components of MetS: waist circumference, HDL, triglycerides, fasting blood glucose, and blood pressure.

Results

Dietary saturated fat and monounsaturated fat, as reported in the DHQ, predicted MetS diagnosis. Dietary monounsaturated fat as reported on the DHQ was directly related to the number of features of MetS. Additionally, the n-3 fatty acids ALA, EPA, and DHA, as reported on the food record were inversely related to features of MetS. Increased levels of plasma 14:0, 16:3n4, 18:1n9, and 20:1n9 20:5n3 were predictive of MetS. Plasma saturated and monounsaturated fatty acids were positively correlated, while plasma polyunsaturated fatty acids were negatively correlated, with triglyceride levels. Plasma ratios of 16:1 to 16:0 and 18:1 to 18:0 were positively correlated with triglyceride levels. The ratio of plasma 18:1 to 18:0 was also negatively correlated to HDL levels. Adipose 16:1n7 and 18:4n3 were decreased in subjects with increasing numbers of MetS features. Adipose 16:0 was correlated with each feature of MetS.
Conclusions

Dietary, plasma, and adipose fatty acid composition were shown to have relationships to components of metabolic syndrome. Specifically, we have shown a relationship between n-3 fatty acid consumption and the presence of MetS components, suggesting a potential role for increased n-3 consumption and the reduction of MetS prevalence.

Key Words

Metabolic syndrome, Older Adults, Fatty Acids
Introduction

The metabolic syndrome (MetS) is a cluster of metabolic disturbances that includes central obesity, dyslipidemia, hypertension, and hyperglycemia. In the 1988-1994 NHANES III survey, MetS was estimated to affect 22% of the adult US population(1). Since then, the prevalence of MetS has increased and is estimated to affect 34% of the adult population, including nearly 50% of males and 57% of females over age 60(2).

Because MetS is associated with an elevated risk of both type 2 diabetes and cardiovascular disease, an increased incidence of MetS predicts for increased morbidity and mortality attributed to these diseases(3). A meta-analysis of 21 studies found that the presence of MetS increases the risk of mortality from all causes by 35%, including a 74% increase in the relative risk of cardiovascular disease and a 76% increase in the relative risk of stroke(4). Similarly, normal weight individuals with MetS have four times the risk of developing type 2 diabetes as those without the syndrome while obese individuals with MetS have 10 times the risk of incident diabetes compared to unaffected, normal weight controls(5). The increased risk of death from MetS also holds true specifically for older adults. A study of an Italian cohort of adults over 65, found an increased risk of all cause mortality (hazard ratio 1.41) and cardiovascular mortality (hazard ratio 1.60) in subjects with MetS(6).
There is no internationally agreed upon definition of MetS. However, in the United States, one common definition is derived from the Adult Treatment Panel III where MetS is defined as having three or more of the following criteria:

1. Waist circumference greater than 103 cm in males or 88 cm in females;
2. HDL less than 40 mg/dL in males or 50 mg/dL in females or drug treatment for low HDL;
3. Triglycerides greater than 150 mg/dL or drug treatment for high triglycerides;
4. Fasting blood glucose greater than 100 mg/dL or drug treatment for high fasting glucose;
5. Blood pressure greater than 130/85 mmHg or drug treatment for high blood pressure.

There is evidence that the composition of dietary fats is related to MetS risk. Specifically, elevated intake of saturated fats had increases the odds of having MetS (8). Dietary laurate (12:0), myristate (14:0), and palmitate (16:0) have been found to raise blood cholesterol levels (9). Conversely, substituting monounsaturated fat for saturated fat in a moderate fat diet has been shown to increase insulin sensitivity (10).

Polyunsaturated fatty acid consumption has been associated with a decrease in the incidence of MetS. A prospective study of over 3000 middle-aged men and women in Korea reported a 67% decrease in MetS incidence in men who consumed fish daily compared to those who ate fish once per week. The intake of fatty fish was most strongly associated with lower triglyceride and increased HDL levels. No effect was seen in women (11). Similarly, a cross-sectional study of 2500 adults in Tehran found an inverse relationship between MetS and both alpha-linolenic acid (ALA, 18:3n3) and total n-6 fatty acid consumption (12).
Plasma fatty acid composition may partially represent the composition of fatty acid intake over several days prior to blood collection. However, plasma fatty acid composition may also reflect endogenous fatty acid metabolism(13). Interestingly, higher levels of plasma saturated and monounsaturated fats and lower levels of linoleic acid (18:2n6), arachidonic acid (20:4n6), and docosahexaenoic acid (DHA, 22:6n3) have been found in those with MetS (14). Consistent with this finding, a separate study found levels of stearic acid (18:0), palmitoleic acid (16:1) and linoleic acid were increased in overweight subjects with MetS when compared to normal weight controls without MetS(15). A separate study of adults in Asia showed similar results: blood levels of total monounsaturated fatty acids, palmitoleic acid and oleic acid (18:1n9) were increased in subjects with increasing numbers of MetS risk factors (16). Investigating individual components of MetS, plasma palmitoleic acid content was positively related to triglyceride levels and negatively associated with HDL levels while linoleic acid and 18:3n6 were negatively correlated with systolic and diastolic blood pressure(17).

Desaturases are enzymes that increase the number of double bonds in fatty acid hydrocarbon chains. As a marker of stearoyl-CoA desaturase activeity, the desaturase index is calculated by finding the ratios of plasma 16:1/16:0 and 18:1/18:0 and may indicate high lipogenic activity(18; 19). Several studies have shown that the desaturase index was higher in subjects with a greater number of metabolic syndrome risk factors and increased insulin resistance (14; 16; 20).
In contrast to plasma fatty acid composition, which is easily influenced by recent fatty acid intake as well as metabolism of fatty acids, the fatty acid composition of adipose tissue may be a better marker of habitual dietary fatty acid intake over the past 12 to 18 months(21). Few studies have been undertaken to investigate the relationship between adipose fatty acid composition and MetS components. A study of adults in Costa Rica found that higher arachidonic acid in gluteal adipose tissue predicted for MetS, even after the model was adjusted for multiple confounders(22). In contrast to findings in plasma that showed that high saturated fatty acid content was positively associated with MetS(14), the myristic acid and stearic acid content of subcutaneous abdominal adipose tissue have been found to be correlated with increasing insulin sensitivity(23).

Based on these observations, our goal in this study was to investigate the relationship between the features of MetS in older adults and the fatty acid composition of the diet as well as plasma and adipose tissue. Data were analyzed by investigating correlations between fatty acid composition and MetS features, examining differences in fatty acid levels between groups of subjects with increasing numbers of MetS features, and discovering which fatty acids best predict for MetS as a whole.

Methods

This study was conducted as part of a larger cross-sectional study of dietary protein sources and markers of health in older persons (24). Subjects between the ages of 60 and 88 were recruited from across Ohio over a seven-month period. Participants were recruited through newspaper, radio and television advertisements and fliers at grocery
stores, public libraries, senior centers and group meal sites. Eligibility criteria for the study included: at least 60 years of age at screening, non-institutionalized, ambulatory, and mentally competent. In addition, they could not report abusing drugs or alcohol, and could not have had surgery in the last three months or have diseases that altered their food intake. Subjects were compensated for their time with a $40 payment. The study was approved by The Ohio State University Institutional Review Board.

*Dietary Intake*

Dietary intake over the past 12 months was assessed with the Diet History Questionnaire (DHQ) developed by the National Cancer Institute of the National Institutes of Health (2004). Subject responses on the questionnaire, which measures the consumption of 124 food items over the past year, were reviewed by study personnel and analyzed by Optimum Solutions Corporation (Lynbrook, NY). Dietary intake was also assessed in a subset of the subjects by a 7-day weighed food record. Subjects received an electronic scale to weigh all foods consumed and were instructed on how to properly use it by study personnel. The day after completion of the record, study personnel reviewed the record with the subject to clarify any ambiguous entries. Dietary records were analyzed using the Nutrition Data System for Research (2005).

*Anthropometric Measurements*

Standing height was measured with a calibrated stadiometer at the end of normal exhalation and rounded to the nearest 0.1 cm. Weight was measured with a calibrated
scale. Subjects were weighed in light clothing without shoes and the value was rounded to the nearest 0.1 kg. Both height and weight were measured twice and the average of the measurements was reported. Waist circumference was measured using an anthropometric tape measure at the narrowest circumference between the costal margin and iliac crest. Subjects were standing upright and were measured at the end of normal exhalation. Waist circumference was measured in triplicate and the average of the measurements was reported.

**Biochemical Measurements**

A qualified nurse collected fasting blood samples from a subset of the subjects (n=56) between 7 and 10 am to minimize diurnal variation. The sample was analyzed for blood lipids (total cholesterol, LDL, HDL, and triglycerides) at The Ohio State University Medical Center by enzymatic assay. Blood pressure was measured by sphygmomanometer. Blood glucose was measured by glucose oxidase method in The Ohio State University Department of Human Nutrition laboratory.

**Plasma Fatty Acid Composition Analysis**

A portion of collected blood was aliquoted for plasma fatty acid analysis. Lipids were extracted with chloroform:methanol (2:1 v:v), and methylated into fatty acid methyl esters which were analyzed by gas chromatography using a 30-m Omegawax capillary column (Supelco Chromotography Products) and helium as a carrier gas. Retention times were compared to authentic standards.
Adipose Fatty Acid Composition Analysis

Subjects provided subcutaneous fat samples at the day after completing their seven-day weighed food records. Samples were taken from the upper left quadrant of the buttock according to a method by Beynen and Katan(25). A qualified nurse used a vacuum tube assembly consisting of an evacuated 10-mL tube, tube holder, connector and 19G needle to collect the sample. Each 35mg sample was immediately frozen and stored at -80 degrees C and protected from light. Samples were pulverized/homogenized and lipids were extracted with chloroform:methanol (2:1 v:v), and methylated into fatty acid methyl esters which were analyzed by gas chromatography using a 30-m Omegawax capillary column (Supelco Chromotography Products) and helium as a carrier gas. Retention times were compared to authentic standards.

Determination of MetS status

Subjects were considered to have MetS if they met the clinical diagnostic criteria for MetS, which are three or more of the following: waist circumference greater than 103 cm in males or 88 cm in females, HDL less than 40 mg/dL in males or 50 mg/dL in females or drug treatment for low HDL, triglycerides greater than 150 mg/dL or drug treatment for high triglycerides, fasting blood glucose greater than 100 mg/dL or drug treatment for high fasting glucose, blood pressure greater than 130/85 mmHg or drug treatment for high blood pressure(7).

Statistical Analysis
Student t-tests for continuous variables and Chi-squared tests for categorical variables were used to determine the significance of differences in subject characteristics by MetS status. Binary logistic regression was used to predict the probability of having MetS or MetS components. Predictor variables included adipose fatty acids 9:0, 14:0, 16:0, 16:1n7, 16:3n4, 17:0, 18:0, 18:1n7, 18:1n9, 18:2n6, 18:3n3, 18:3n4, 18:4n3, 20:1n9, 20:4n3, 20:4n6, 20:5n3, 22:5n3, 22:6n3, and plasma fatty acids 9:0, 14:0 16:0, 16:1n7, 17:0, 16:3n4, 18:0, 18:1n9, 18:1n7, 18:2n6, 18:3n3, 18:3n4, 18:4n3, 20:1n9, 20:4n6, 20:4n3, 20:5n3, 22:5n3, 22:6n3. Dietary components were also predictor variables as reported on the DHQ and food record and analyzed per 1000 kcals consumed. They included: protein, carbohydrates, saturated fat, monounsaturated fat, polyunsaturated fat, cholesterol, fiber, Vitamin A, and Vitamin C. Correlation analysis using Kendall's tau coefficient was used to explore relationships between predictor variables and components of Mets. Individual predictors were used to predict the probability of MetS or its components. A stepwise approach was used to find models with several predictors. Subjects were categorized into groups if they had 0, 1-2 or 3-5 components of MetS and ANOVA with Tukey's multiple comparisons was used to find differences between groups. Statistical analysis was performed using SAS software (version 9.2; SAS Institute Inc, Cary, NC) with significance at $\alpha=0.05$. 

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Results

Subject Characteristics

Of the 165 subjects consented, 142 completed the study. Five withdrew due to health problems unrelated to the study, 7 exited due to lack of interest or time, 8 were lost to follow up, and 3 provided incomplete data. The average age at the time of the study was 73.3 +/- 6.7 years. 67% of the subjects were female and 86% were Caucasian. 97% of the subjects had achieved at least a high school diploma while 60% had earned a bachelor's degree. Most subjects were married (56%) or widowed (28%) and 62% lived with others. These demographics did not differ significantly between those with and without MetS (Table 1).

Forty-two subjects had at least 3 of the 5 characteristics of MetS and therefore met the criteria for MetS. Thirty-three of the subjects did not meet the diagnostic criteria for MetS. Blood was not drawn to determine fasting blood glucose and blood lipids on the remaining subjects, so they were not included in the analysis. The average values of the features of MetS in each group are given in Table 1. Triglyceride, glucose, HDL, and waist circumference levels differed significantly between those with and without MetS (p<0.001). Of those with MetS, 69% had low LDL, 61.7% had high blood pressure, 49.3% had high fasting glucose, 46.9% had an increased waist circumference, and 21% had high triglycerides, according to the criteria given above (data not shown).
Dietary intake and MetS

Dietary saturated fat and monounsaturated fat, as reported in the DHQ and controlled per 1000 calories consumed, predicted for having MetS (Table 2A). The intake of other macronutrients as well as intake of Vitamins A and C did not predict for having MetS. Dietary monounsaturated fat as reported in the DHQ and the n-3 fatty acids ALA, EPA, and DHA, as reported on the FR, were found to be related to the number of features of MetS (Figure 1).

Plasma fatty acid composition and MetS

Increased levels of plasma 14:0, 16:3n4, 18:1n9, 20:1n9, and 20:5n3 predicted for having MetS (Table 2B). Individual plasma saturated and monounsaturated fatty acids were positively correlated, while individual plasma polyunsaturated fatty acids were negatively correlated, with triglyceride levels. Two exceptions to this observation are 16:3n4, a peroxisome-derived product that was positively correlated with triglyceride levels, and 17:0 which was negatively correlated with triglyceride levels (Table 3A).

Plasma ratios of 16:1 to 16:0 and 18:1 to 18:0 in the plasma, which may be used as markers of SCD activity, were not related to meeting the criteria for MetS. However, these ratios were positively correlated with triglyceride levels (p<0.0001). The ratio of 18:1 to 18:0 was also negatively correlated with HDL levels (p=0.01, Table 3A).
Adipose fatty acid composition and MetS

Gluteal adipose 16:1n7 and 18:4n3 was decreased, in subjects with increasing numbers of MetS features (Figure 2). In addition, adipose 16:0 was correlated with each feature of MetS (Table 3B). However, none of the adipose fatty acids predicted for MetS as a whole.

Discussion

In healthy older adults from Ohio, the fatty acid composition of the diet, plasma, and adipose tissue appeared to differ between subjects with and without MetS. Plasma fatty acid composition was correlated with triglyceride levels, with plasma saturated and monounsaturated fat being positively correlated and plasma unsaturated fat being negatively correlated. The ratios of 16:1/16:0 and 18:1/18:0 were also positively correlated with triglyceride levels. Interestingly, adipose 16:0 correlated with each feature of MetS.

The characteristics of the subjects in our study were similar, but not identical, to that of the national and Ohio older adult population. Caucasians made up 86% of our cohort being white compared to 89% of the Ohio older adult population and 82.4% nationally. Our group was less likely to be married and more likely to be widowed than the national population (56% married in our cohort vs 72-79% nationally and 58.3% in Ohio). Finally, our cohort was more educated than the general older adult population with 97% of our population achieving a high school diploma and 60% earning a bachelor’s degree.
compared to 78% and 21%, respectively, in the national older adult population and 68.5% earning a high school degree in Ohio (26; 27). We found that 55% of the subjects met the diagnostic criteria for metabolic syndrome. This is consistent with the reported national average for this age group of 50-57% (2).

As might be expected, we found that saturated fat consumption predicts for having MetS. Numerous studies have reported that diets high in saturated fat are associated with lower insulin sensitivity and an increased risk of MetS (13; 28; 29). The positive relationship between dietary intake of monounsaturated fats with MetS components, however, was unexpected. In contrast to previous studies, we found a relationship between higher monounsaturated fat consumption and the number of MetS features. At least one randomized controlled trial has found a beneficial effect of increased monounsaturated fat consumption on insulin sensitivity, especially when total dietary fat accounts for less than 37% of total calorie intake, as it did in our study (10). Studies showing the beneficial effect often substitute vegetable-based oils for saturated fat from animal products (10; 30; 31). However, in the typical diet of American adults, the largest source of monounsaturated fats is beef, accounting for 11.4% of consumption. Oils account for the second highest food source at 8.4% (32). Therefore, monounsaturated fat consumption in our study might not be from oils, but from beef and other animal foods, which also contain saturated fatty acids.

Dietary n-3 fatty acids were associated with the number of MetS components, with lower intakes being found in those with the most features of MetS. Our findings are consistent
with two previous studies conducted in younger populations where high fish or ALA consumption was associated with a decreased risk of MetS (11; 12). However, these studies were conducted in Korea and Tehran, respectively, where dietary patterns are likely quite different from those in Ohio.

Plasma 18:1n9 predicted for MetS in our older adult population, and our finding is in agreement with previous reports in younger adults (14). However, it had not previously been shown that so many fatty acids correlate with triglyceride levels. Supplementation with n-3 fatty acid has previously been shown to reduce triglyceride levels (13). In our study, n-3 plasma fatty acid composition did not correlate with triglyceride levels. As n-3 fatty acids were not supplemented in this observational study, it is possible that n-3 levels were too low in our cohort to affect blood triglycerides.

An increased SCD index in the plasma may predict for MetS (3; 14). Although we did not find that these ratios predicted MetS as a whole, we did find a positive relationship with triglyceride levels and a negative relationship with HDL levels. To our knowledge, we are the first to show this relationship in human plasma, although a negative association between hepatic SCD mRNA and HDL levels has been recently reported in mice (33).

Few studies have been undertaken to examine fatty acid composition of the adipose tissue and MetS risk. To our knowledge, we are the first to find that adipose palmitic acid (16:0) composition is significantly correlated with each aspect of MetS. Palmitic acid consumption may raise cholesterol levels (9), and increased serum levels of palmitic acid
are associated with an increased odds of having coronary heart disease and diabetes (34; 35). Therefore, it is not surprising that adipose levels of palmitic acid are positively related to risk factors for heart disease including blood pressure, high triglycerides, low HDL, and elevated fasting glucose.

This study has demonstrated relationships of dietary, plasma, and adipose fatty acid composition with risk factors for metabolic syndrome. Most importantly, we have shown an inverse relationship between n-3 fatty acid consumption and the presence of MetS components, suggesting a potential role for increased n-3 consumption and the reduction of MetS prevalence in older adults. Further research should be undertaken to better determine the relationship between n-3 fatty acids and MetS in a larger cohort. In addition, a randomized controlled trial should be conducted to determine whether supplementation with n-3 fatty acids, either from oils or from foods, might improve markers of MetS.
References


27. Scripps Gerontology Center. Profile and projections of the 60+ population Ohio [Internet]. Available from: http://www.scripps.muohio.edu/sites/scripps.muohio.edu/files/ohio_005.pdf


Table 1- Characteristics of subjects with and without MetS.

<table>
<thead>
<tr>
<th></th>
<th>Met S (n=33)</th>
<th>No MetS (n=42)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Stdev</td>
<td>Mean</td>
</tr>
<tr>
<td>Age (years)</td>
<td>71.5</td>
<td>6.38</td>
<td>73.76</td>
</tr>
<tr>
<td>% Female</td>
<td>66.67</td>
<td>--</td>
<td>66.67</td>
</tr>
<tr>
<td>% Caucasian</td>
<td>78.57</td>
<td>--</td>
<td>90.91</td>
</tr>
<tr>
<td>% Married</td>
<td>61.90</td>
<td>--</td>
<td>51.52</td>
</tr>
<tr>
<td>% College Degree</td>
<td>52.34</td>
<td>--</td>
<td>63.64</td>
</tr>
<tr>
<td>% Living with Others</td>
<td>66.67</td>
<td>--</td>
<td>57.58</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>31.94</td>
<td>4.35</td>
<td>26.09</td>
</tr>
<tr>
<td>Systolic (mmHg)</td>
<td>135.7</td>
<td>17.35</td>
<td>133.24</td>
</tr>
<tr>
<td>Diastolic (mmHg)</td>
<td>73.8</td>
<td>10.28</td>
<td>72.39</td>
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<tr>
<td>HDL (mg/dL)</td>
<td>43.48</td>
<td>9.79</td>
<td>56.72</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>128.48</td>
<td>51.61</td>
<td>84.5</td>
</tr>
<tr>
<td>Fasting Glucose (mg/dL)</td>
<td>109.38</td>
<td>20.52</td>
<td>91.94</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>103.71</td>
<td>12.31</td>
<td>85.85</td>
</tr>
</tbody>
</table>
Table 2- Fat content and risk of MetS

Dietary fat intake as reported on the diet history questionnaire (DHQ, A) and Plasma Fatty Acid content (B) predict for MetS.

A.

<table>
<thead>
<tr>
<th>DHQ intake (per 1000 kcal)</th>
<th>Estimate</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated Fat</td>
<td>0.1890</td>
<td>0.0290</td>
</tr>
<tr>
<td>Monounsaturated Fat</td>
<td>0.2364</td>
<td>0.0137</td>
</tr>
<tr>
<td>Polyunsaturated Fat</td>
<td>0.0854</td>
<td>0.4381</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.0060</td>
<td>0.2578</td>
</tr>
<tr>
<td>Protein</td>
<td>0.0387</td>
<td>0.2446</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>-0.0229</td>
<td>0.0630</td>
</tr>
<tr>
<td>Dietary Fiber</td>
<td>-0.1168</td>
<td>0.1843</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>-0.0001</td>
<td>0.3125</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>-0.0110</td>
<td>0.1189</td>
</tr>
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</table>

Continued
Table 2 Continued

B.

<table>
<thead>
<tr>
<th>Plasma Fatty Acid</th>
<th>Estimate</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>4.0844</td>
<td>0.0129</td>
</tr>
<tr>
<td>16:0</td>
<td>0.2530</td>
<td>0.365</td>
</tr>
<tr>
<td>16:1n7</td>
<td>0.8730</td>
<td>0.0769</td>
</tr>
<tr>
<td>16:3n4</td>
<td>25.7350</td>
<td>0.0072</td>
</tr>
<tr>
<td>18:0</td>
<td>0.1335</td>
<td>0.6521</td>
</tr>
<tr>
<td>18:1n9</td>
<td>0.1891</td>
<td>0.0411</td>
</tr>
<tr>
<td>18:2n6</td>
<td>0.0204</td>
<td>0.6961</td>
</tr>
<tr>
<td>18:3n3</td>
<td>2.4739</td>
<td>0.2304</td>
</tr>
<tr>
<td>18:4n3</td>
<td>0.2664</td>
<td>0.6652</td>
</tr>
<tr>
<td>20:1n9</td>
<td>17.5407</td>
<td>0.0432</td>
</tr>
<tr>
<td>20:4n6</td>
<td>-0.0681</td>
<td>0.3893</td>
</tr>
<tr>
<td>20:5n3</td>
<td>-1.2554</td>
<td>0.0371</td>
</tr>
<tr>
<td>22:6n3</td>
<td>-0.0479</td>
<td>0.9113</td>
</tr>
<tr>
<td>Total n-3</td>
<td>-0.4372</td>
<td>0.1086</td>
</tr>
<tr>
<td>Total n-6</td>
<td>-0.0098</td>
<td>0.8517</td>
</tr>
<tr>
<td>N-3/N-6</td>
<td>-12.4202</td>
<td>0.1218</td>
</tr>
</tbody>
</table>
Table 3- Plasma and adipose fatty acids and MetS components
Plasma fatty acids (A) and adipose fatty acids (B) correlate with the features of MetS
* p< 0.05 ** p<0.01

A.

<table>
<thead>
<tr>
<th>Plasma Fatty Acid (%)</th>
<th>Systolic BP</th>
<th>Diastolic BP</th>
<th>Triglycerides</th>
<th>HDL</th>
<th>Glucose</th>
<th>Waist</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:0</td>
<td>-0.1248</td>
<td>0.0142</td>
<td>-0.1008</td>
<td>0.1614</td>
<td>-0.0791</td>
<td>-0.2443*</td>
</tr>
<tr>
<td>14:0</td>
<td>0.2043</td>
<td>0.1889</td>
<td>0.3446**</td>
<td>-0.0279</td>
<td>0.2520*</td>
<td>0.2105*</td>
</tr>
<tr>
<td>16:0</td>
<td>0.1998</td>
<td>0.1101</td>
<td>0.2802**</td>
<td>0.0615</td>
<td>0.1691</td>
<td>0.1169</td>
</tr>
<tr>
<td>16:1n7</td>
<td>0.2534*</td>
<td>0.0495</td>
<td>0.3869**</td>
<td>0.0749</td>
<td>0.1109</td>
<td>0.1370</td>
</tr>
<tr>
<td>17:0</td>
<td>-0.1331</td>
<td>-0.1249</td>
<td>-0.2952*</td>
<td>0.1090</td>
<td>-0.0577</td>
<td>0.0513</td>
</tr>
<tr>
<td>16:3n4</td>
<td>0.3360**</td>
<td>0.0652</td>
<td>0.4136**</td>
<td>-0.0235</td>
<td>0.1893</td>
<td>0.2283*</td>
</tr>
<tr>
<td>18:0</td>
<td>0.0636</td>
<td>0.0157</td>
<td>0.00445</td>
<td>0.2111*</td>
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<tr>
<td>18:1n9</td>
<td>0.2266*</td>
<td>0.0900</td>
<td>0.4025**</td>
<td>-0.1240</td>
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<td>0.0926</td>
<td>0.0090</td>
<td>0.2135*</td>
<td>-0.0838</td>
<td>-0.1198</td>
<td>0.0991</td>
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<tr>
<td>18:2n6</td>
<td>-0.0234</td>
<td>-0.0562</td>
<td>-0.2401*</td>
<td>-0.1151</td>
<td>-0.1669</td>
<td>0.0278</td>
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<tr>
<td>18:3n4</td>
<td>0.0635</td>
<td>0.0978</td>
<td>0.1433</td>
<td>0.0204</td>
<td>-0.0337</td>
<td>0.1160</td>
</tr>
<tr>
<td>18:3n3</td>
<td>-0.0525</td>
<td>-0.0810</td>
<td>0.1423</td>
<td>-0.0101</td>
<td>-0.0907</td>
<td>-0.0100</td>
</tr>
<tr>
<td>18:4n3</td>
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<td>0.0292</td>
<td>-0.1223</td>
<td>-0.1039</td>
<td>-0.2161</td>
<td>0.0189</td>
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<tr>
<td>20:1n9</td>
<td>0.1775</td>
<td>0.1529</td>
<td>0.2824**</td>
<td>-0.1397</td>
<td>0.0952</td>
<td>0.2105*</td>
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<tr>
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<td>0.0247</td>
<td>-0.2535*</td>
<td>0.0212</td>
<td>0.0840</td>
<td>-0.0679</td>
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<tr>
<td>20:4n3</td>
<td>-0.1605</td>
<td>0.0147</td>
<td>0.0134</td>
<td>-0.0753</td>
<td>0.0214</td>
<td>-0.0862</td>
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<tr>
<td>20:5n3</td>
<td>-0.0882</td>
<td>0.0450</td>
<td>-0.1156</td>
<td>0.2022</td>
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<td>0.0534</td>
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<td>-0.1418</td>
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<td>0.1754</td>
<td>-0.0482</td>
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<tr>
<td>16:1/16:0</td>
<td>0.19</td>
<td>-0.02</td>
<td>0.34**</td>
<td>0.12</td>
<td>0.01</td>
<td>0.05</td>
</tr>
<tr>
<td>18:1/18:0</td>
<td>0.16</td>
<td>0.07</td>
<td>0.43**</td>
<td>-0.29*</td>
<td>0.17</td>
<td>0.18</td>
</tr>
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</table>

Continued
Table 3 continued
B.

<table>
<thead>
<tr>
<th>Adipose Fatty Acid (%)</th>
<th>Systolic BP</th>
<th>Diastolic BP</th>
<th>Triglycerides</th>
<th>HDL</th>
<th>Glucose</th>
<th>Waist</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:0</td>
<td>0.1742</td>
<td>-0.0278</td>
<td>0.1518</td>
<td>-0.0808</td>
<td>0.1223</td>
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<tr>
<td>14:0</td>
<td>0.3356*</td>
<td>0.2278</td>
<td>-0.15615</td>
<td>-0.2536</td>
<td>0.1296</td>
<td>0.1537</td>
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<tr>
<td>16:0</td>
<td>0.4015**</td>
<td>0.2001</td>
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<td>0.4054**</td>
<td>-0.2515</td>
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</tr>
<tr>
<td>16:3n4</td>
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<td>-0.1715</td>
<td>0.1183</td>
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</tr>
<tr>
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<td>0.1449</td>
<td>-0.0032</td>
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Figures

Figure 1- Dietary fatty acids and MetS features present
(A) Dietary monounsaturated fat (MUFA) intake as reported on the diet history questionnaire (DHQ) differs in individuals with increasing numbers of MetS features. A-
linolenic acid (18:3n3, B), EPA (20:5n3, C) and DHA (22:6n3, D) as reported on the 7-
day weighed food record (food record) differ in individuals with increasing numbers of
MetS features.

A.
Figure 1 continued

B.

C.
Figure 1 continued

D.

![Bar chart showing the percentage of food records 22.6m3 across different features of MetS present.](chart)

- p=0.005, p=0.007
Figure 2- Adipose fatty acids and MetS features present
Adipose fatty acid composition of 16:1n7 (A) and 18:4n3 (B) decrease in subjects with increasing features of MetS.

A.

B.
Chapter 4: Epilogue

MetS is a common condition that affects over half of Americans over the age of 60 and leads to an increased risk of diabetes and heart disease. In a cohort of older adults, we found that the fatty acid composition of the diet, especially n-3 fatty acids, differs among those with increasing numbers of metabolic syndrome features. Plasma fatty acid composition was correlated with triglyceride levels with plasma saturated and monounsaturated fat generally being positively correlated and plasma unsaturated fat generally being negatively correlated. The ratios of 16:1/16:0 and 18:1/18:0 were also positively correlated with triglyceride levels. Finally, adipose 16:0 correlated with each feature of MetS.

Although more research needs to be done, the relationships seen in this observational study suggests the possibility that the risk of MetS and its components may be modifiable by making dietary changes or altering fatty acid metabolism. This is important because the prevalence of MetS continues rise. Although the component problems of MetS are pharmaceutically treatable, the increased risk of mortality and serious morbidity remains and the drugs used to treat the features of MetS are not without side effects. Therefore it is important to find specific lifestyle changes that patients can make to reduce their risk of MetS. Although merely observational at this point, this study suggests that increasing consumption of n-3 fatty acids while decreasing consumption of saturated fatty acids like 16:0 might help decrease the risk of developing features of MetS in older adults.
This study, however, has some limitations. First, it was limited by sample size. Although the original sample was large, only 75 of the original 142 subjects gave enough data to be included in our analysis. A larger sample size might increase our power to determine relationships between fatty acids and MetS. It would also enable more complex statistical analysis like controlling for confounders and performing multivariate analysis.

In addition, most studies involving dietary measurements are limited by relying on self-report which may be biased. Subjects have been shown to often under-report food consumption on both DHQs and food records\(^5^3\). Weighed food records, like the one used in this study, may help eliminate some of this bias, but increase the burden on the subjects in the trial. One solution to this problem is observing the subjects eating their usual diet, but this also increases the burden on the subjects while greatly increasing the workload for study personnel. Another solution is using plasma and adipose fatty acid composition as markers of dietary intake as we did in this study. However, both are imperfect markers of fatty acid consumption due to alterations some fatty acids undergo once inside the body.

Finally, this study was limited by its cross-sectional design. Because the study was not a randomized controlled trial, causality could not be determined, and we do not know if the relationships between fatty acid composition and MetS components are causal or merely co-occurring states. In particular, the nature of the relationship between plasma fatty acid composition and MetS is unclear. Plasma fatty acid composition reflects both dietary intake and fatty acid metabolism. Because of this, we do not know if relationships
between plasma fatty acids and features of MetS are a result of diet, metabolic
differences between subjects, or a combination of the two.

In order to determine if the relationship between fatty acid composition and MetS
components is a causal relationship, future studies should be conducted to randomize the
consumption of different types of fat. In particular, 16:0 and n-3 fatty acids are of interest
for their relationship to MetS components and their potential for decreasing the risk of
MetS.
References


