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Roles of Serum Lycopene in the Prevalence and Mortality of Metabolic Syndrome in the Adult Population

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ROLES OF SERUM LYCOPENE IN THE PREVALENCE AND MORTALITY OF METABOLIC SYNDROME IN THE ADULT POPULATION

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Metabolic syndrome (MetS) is a cluster of metabolic disorders, including increased fasting glucose, blood pressure, plasma triglyceride, reduced high-density lipoprotein cholesterol and abdominal obesity. It leads to an increased risk of cardiovascular disease and diabetes. The growing prevalence of MetS is strongly related to the increasing prevalence of overweight/obesity. As an antioxidant, lycopene can reduce the risk of MetS. However, it is unclear whether lycopene has similar effects among overweight/obese individuals and whether lycopene can reduce the risk of mortality among individuals with MetS. The purpose of this study was to explore the roles of lycopene in the prevalence and mortality of MetS. Specifically, the main objective was to examine the associations between serum lycopene or the ratio of serum lycopene to serum triglyceride and MetS, and the association between the ratio and mortality among individuals with MetS. In addition, the possible additive effects of physical activity and lycopene on MetS and mortality were studied. To achieve these objectives, analyses were conducted with participants aged 20 years and older from the NHANES 2001-2006. The tertile rank method was used to divide participants into three groups according to serum lycopene or the ratio. Logistic regression and Cox models were used for association analyses. With serum lycopene, the associations between lycopene and MetS were only significant for normal weight/overweight ($p<0.05$), but not for obese participants ($p>0.05$). While with the ratio, the associations between lycopene and MetS were significant not only for normal weight/overweight ($p<0.05$), but also for obese participants ($p<0.05$). Compared with the first tertile group, both the third

and second tertile groups had significantly reduced hazard ratios of mortality for participants with MetS. The additive effect of lycopene and physical activity was significant for overweight ($p<0.05$) but not for obese participants ($p>0.05$). There was no an additive effect of lycopene and physical activity on mortality among participants with MetS. Therefore, the study adds new evidence that the ratio of serum lycopene to serum triglyceride has significant associations with morbidity and mortality of MetS.

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BMI	Body mass index
CI	Confidence Intervals
DNA	Deoxyribonucleic acid
DM	Diabetes mellitus
HDL	High-density lipoprotein
HR	Hazard ratio
IL-6	Interleukin-6
IL-8	Interleukin-8
IL-10	Interleukin-10
MEC	Mobile examination center
MetS	Metabolic syndrome
NHANES	National Health and Nutrition Examination Survey
OR	Odds ratio
PPS	Probabilities proportionate to the measure of size
PSU	Primary sampling units
TNF-α	Tumor necrosis factor-alpha
WHO	World Health Organization

CHAPTER 1 INTRODUCTION

1.1. Metabolic Syndrome

Metabolic syndrome is a cluster of metabolic disorders, including increased fasting glucose, blood pressures, plasma triglyceride, and reduced high-density lipoprotein (HDL) cholesterol concentrations and abdominal obesity [1]. According to the National Cholesterol Education Program's Adult Treatment Panel III, to be diagnosed with metabolic syndrome, an individual has to meet three or more of the following criteria: abdominal obesity (waist circumference>40 inches for men and>35 inches for women); hypertriglyceridemia (serum triglyceride≥1.69 mmol/L); low HDL-cholesterol (HDL<1.04 mmol/L for men and<1.29 for women); hypertension (≥130/85 mmHg) and high fasting glucose (fasting glucose≥6.1 mmol/L) [2]. With the diagnosis criteria, approximately 27% of adults, aged 20 years and older, had metabolic syndrome in 2000 based on the National Health and Nutrition Examination Survey (NHANES) 1999–2000 [2]. Metabolic syndrome is common among overweight and obese individuals, and leads to an increased risk of cardiovascular disease and type 2 diabetes mellitus [1, 2]. Additionally, metabolic syndrome is associated with increased risks for certain cancers, including breast [3], endometrial [4], colorectal [5] and biliary tract cancers [6], and an increased risk for all-cause mortality [7, 8]. Thus, given the number of affected individuals and the associated disease risks, metabolic syndrome is an important public health concern in the United States.

Although the mechanisms behind metabolic syndrome are not entirely clear, accumulating evidence supports that chronic inflammation and oxidative stress play important roles in its development [9, 10]. For example, increased body mass index (BMI) is found to increase inflammation and oxidative stress production [11-13]. Also, the prevalence of

metabolic syndrome strongly correlates with an increased prevalence of overweight and obesity [11-13].

1.2. Health Effects of Lycopene

There have been a number of different strategies or interventions intended to mitigate the oxidative stress and/or inflammation. One is the therapeutic drug strategy. For example, salsalate, a traditional anti-inflammatory medication, can prevent the metabolic syndrome in obese non-diabetic individuals [14]. Probucol, a potent anti-oxidant drug, has been in clinical use during the past few decades for prevention of cardiovascular diseases [15]. While these therapeutic drug strategies that target weight-related oxidative stress and/or inflammation can potentially reduce chronic disease burdens, often these anti-oxidants and anti-inflammatory drugs have serious side effects in long-term disease prevention. For example, approximately 30% of patients discontinued use of salsalate because of gastrointestinal symptoms and tinnitus [16] and many patients discontinued use of probucol because of the space between the start of the Q wave and the end of the T wave (Q-T interval) prolongation [17].

A diet rich in antioxidants is another potential approach to reduce oxidation and inflammation. Therefore, nutrient intake from food, such as lycopene, may be an ideal option for long-term disease prevention. As a nutrient, lycopene is mainly contained in tomatoes and other vegetables or fruits, such as red carrots and watermelons. The biological mechanisms of lycopene on metabolic syndrome and chronic diseases include alleviating oxidative stress and inhibiting inflammation in the body [18-22]. Studies found there is a significant association between metabolic syndrome and lycopene [23-25]. For example, Sluijs et al, found that higher lycopene intake is associated with a lower prevalence of metabolic syndrome in middle-aged and elderly men [23]. Liu et al. found that higher serum lycopene levels were associated with a lower prevalence of metabolic syndrome in

middle-aged and elderly Chinese adults [24]. Yeo et al. also found there is a significantly positive association between metabolic syndrome and serum lycopene among Korean men [25]. Further, epidemiologic studies have shown that lycopene can significantly reduce the risks of chronic diseases. For example, since the first report that lycopene can reduce the incidence rate of prostate cancer in 1995 [26], lycopene has been found to be inversely associated with breast, cervical, ovarian, liver and other organ sites cancers [27]. In addition to tumor-suppressing activity, lycopene is found to decrease the risks of developing other chronic diseases, such as cardiovascular disease [28-30] and cerebrovascular disease [31].

Despite accumulating evidence to support health effects of lycopene, it is not clear whether lycopene has similar effects among individuals who are overweight or obese. Many recent studies on lycopene have focused on people who are overweight or obese [32-37]; however, the results remain inconclusive. For example, some studies found that lycopene intervention can significantly reduce inflammation and oxidative stress in participants who are overweight and obese [32-35], while other studies did not find significant associations between lycopene and inflammation and oxidative stress in overweight or obese individuals [36, 37].

One of the potential reasons for varying effects of lycopene is differences in the amount of serum lycopene in these studies. Two cell culture studies found a dose-dependent relationship between the serum lycopene concentration and the amount of reduction in inflammation and oxidative stress [38, 39]. These findings explain non-significant results of lycopene studies when the serum concentration was notably lower. Another point to consider when interpreting the research findings on lycopene is variations in the level of oxidation and inflammation in different individuals. By definition, compared to normal weight individuals, overweight and obese individuals should have higher levels

of oxidation and inflammation. It is possible that among overweight and obese individuals with already high oxidation and inflammation levels, a substantially larger amount of lycopene needs to be applied in order to have observable changes. This may indicate that the lycopene effect on oxidation and inflammation is not linear; there may be some thresholds in the BMI levels (as indicators of existing inflammation and oxidation levels) at which points, considerably more lycopene needs to be added as nutrients before its positive effects can be observed.

A triglyceride is an ester consisting of one glycerol molecule bonded with three fatty acid molecules. As a serum lipid, it helps enable the bidirectional transference of adipose fat and blood glucose from the liver. There is a strong association between serum triglyceride and BMI statuses [40]. Furthermore, serum concentration of triglyceride is significantly associated with increased oxidative stress [41] and increased levels of inflammation [42]. Therefore, serum triglyceride can be regarded as an indicator of existing inflammation and oxidation levels in the body.

Taken together, health effects of serum lycopene may depend not only on serum concentrations, but also on the amount of inflammation and/or oxidative stress in the body. Therefore, the ratio of the serum concentration of lycopene to the amount of inflammation and/or oxidative stress can better predict the association between lycopene and positive health effects than with only the serum concentration of lycopene. There is no method to directly measure the amount of inflammation and/or oxidative stress in the body. Given that serum triglyceride is an indicator of existing inflammation and oxidation levels in the body, this study used serum triglyceride to represent the amount of inflammation and/or oxidative stress.

1.3. Roles of Physical Activity

Physical activity has been shown to prevent metabolic syndrome for individuals who are overweight and obese [43-45]. The biological mechanisms by which physical activity prevents metabolic syndrome include reducing abdominal adipose tissue, serum concentration of triglyceride, and blood pressure, and increasing high-density lipoprotein and insulin sensitivity [46]. In addition, physical activity can prevent metabolic syndrome by decreasing inflammation and inducing an anti-inflammatory environment [47, 48]. For example, physical activity can simultaneously decrease inflammatory cytokines, such as TNF-α and interleukin-6, and increase anti-inflammatory cytokines, such as interleukin-10 [49-51].

There is a similarity in the way lycopene and physical activity both impact metabolic syndrome. For example, both decrease inflammatory cytokines like TNF-α and interleukin-6 [20, 21 and 52] and increase anti-inflammatory cytokines like interleukin-10 [53, 54]. Due to common biological mechanisms by which physical activity and serum lycopene reduce the risk of metabolic syndrome by regulating inflammatory cytokines, it is natural to posit that there will be an additive effect between physical activity and serum lycopene on metabolic syndrome. Although both physical activity and lycopene can reduce the risk of morbidity and mortality of metabolic syndrome, studies suggest that the effects of physical activity or lycopene alone are insufficient to substantially reduce the risk of metabolic syndrome for overweight and obese individuals [44]. Examining the additive effect between physical activity and serum lycopene is important for further reducing the risk of metabolic syndrome among individuals who are overweight and obese.

1.4. Potential Effects of Lycopene on Mortality

As mentioned above, metabolic syndrome has an increased risk of all-cause mortality as well as cardiovascular related mortality [7, 8]. Although the reasons for the higher mortality are not entirely clear, accumulating epidemiologic evidence supports that higher mortality may be largely attributable to increased risks of cardiovascular disease, diabetes and stroke-related morbidity and mortality in individuals with metabolic syndrome [8, 55 and 56]. There are more inflammation and oxidative stress in individuals with metabolic syndrome than individuals without metabolic syndrome [57]. Increased inflammation and oxidative stress lead to the increased risk of cardiovascular disease, diabetes and stroke [58-60].

Past epidemiologic studies suggest that lycopene can reduce the risk of cardiovascular diseases [28-30] and stroke [31]. In addition, lycopene was found to reduce the mortality in the general population [61, 62], in patients with Alzheimer's disease, and in patients with obstructive lung function [63, 64]. However, some intervention studies did not find significant associations between lycopene and cardiovascular diseases [65, 66] and mortality [67]. Therefore, results on the beneficial role of lycopene as an antioxidant in the prevention of cardiovascular diseases and mortality remain conflicted [68]. Although all studies claimed that serum concentration of lycopene increased, high increase of serum lycopene at the baseline (0.371umol/L for women and 0.258 umol/L for men) had a significant positive effect on mortality [61] while a small increase of serum lycopene at the baseline (0.16 μmol/L) had no significant positive effect on mortality [67]. Therefore, different criteria for change in serum concentration of lycopene may be an alternative explanation for these inconsistent results. For this reason, the ratio of the serum concentration of lycopene to the amount of inflammation and/or oxidative stress (relative serum

concentration of lycopene) was used to predict the association between lycopene and mortality.

1.5. Effects of Physical Activity on Mortality

A study found that physical activity is significantly associated with reduced mortality in patients with metabolic syndrome [69]. In this prospective study of more than 13,000 men and women with metabolic syndrome, after 10 years following-up, the authors found that individuals with metabolic syndrome who reported high levels of physical activity at baseline were at a reduced risk of death from all causes compared to those who reported no physical activity [69]. However, metabolic syndrome has a high risk of all-cause mortality as well as cardiovascular related mortality [7, 8]. Therefore, to maximize the reduction of deaths among individuals with metabolic syndrome, it is necessary to explore whether a combination of physical activity and lycopene has a stronger effect on mortality than physical activity or lycopene alone.

1.6. Summary of Research Gaps

Gap 1: Lycopene can reduce the risk of metabolic syndrome by alleviating oxidative stress and inhibiting inflammation; the health effects of lycopene on inflammation and oxidative stress are not significant for obese individuals; the question is whether the health effect of lycopene on metabolic syndrome is significant among obese individuals.

Gap 2: Due to the fact that the effects of lycopene depends on the serum concentration of lycopene and the amount of inflammation and oxidative stress, the question is whether the ratio of serum lycopene to the amount of inflammation and oxidative stress can better predict the health effects of lycopene.

Gap 3: Metabolic syndrome has an increased risk of mortality, lycopene can reduce the risk of mortality in general population and some patients, and the question is whether lycopene can reduce the risk of mortality among individuals with metabolic syndrome.

Gap 4: Although both physical activity and lycopene can reduce the risk of metabolic syndrome, both alone are insufficient for the risk. The question is whether physical activity and lycopene have an additive health effect on metabolic syndrome.

Gap 5: Although both physical activity and lycopene can reduce the risk of mortality of metabolic syndrome, both alone are insufficient for the risk. The question is whether physical activity and lycopene have an additive health effect on mortality.

1.7. Study Purpose and Specific Aims

The purpose of this study was to explore the roles of lycopene on the morbidity and mortality of metabolic syndrome by using a large population-based database. There were five specific aims.

Specific Aim 1: To examine the association between serum concentration of lycopene and the prevalence of metabolic syndrome for individuals with different BMI levels. Hypothesis: The association between serum lycopene and metabolic syndrome is influenced by different BMI levels.

Specific Aim 2: To examine the association between the ratio of serum lycopene to serum triglyceride and the prevalence of metabolic syndrome for individuals with different BMI levels. The association between the ratio of serum lycopene to serum triglyceride and metabolic syndrome is influenced by different BMI levels.

Specific Aim 3: To examine the effect of lycopene on mortality in individuals with metabolic syndrome. Hypothesis: Serum lycopene can reduce the risk of mortality for individuals with metabolic syndrome.

Specific Aim 4: To examine the possible additive effect of physical activity and serum lycopene on the prevalence of metabolic syndrome among overweight and obese individuals.

Specific Aim 5: To examine the possible additive effect of physical activity and serum lycopene on mortality among individuals with metabolic syndrome.

1.8. Theoretical Framework

An increased risk of metabolic syndrome occurs in overweight/obese individuals due to increased chronic inflammation and oxidative stress [11-13]. Lycopene, as a natural antioxidant, can alleviate oxidative stress and inhibit inflammation [18-22]. Therefore, increased serum lycopene has the potential to decrease the risk of metabolic syndrome in individuals who are overweight and obese.

However, the health effects of lycopene on reducing inflammation and oxidative stress in individuals who are overweight and obese remain inconclusive [32-37]. Health effects of serum lycopene may depend not only on serum concentrations, but also on the amount of inflammation and/or oxidative stress in the body. Therefore, the ratio of the serum concentration of lycopene to the amount of inflammation and/or oxidative stress can better predict the association between lycopene and positive health effects than serum concentration of lycopene alone can. There is no method to directly measure the amount of inflammation and/or oxidative stress in the body. Given the strong associations between oxidative stress and inflammation, this study used serum triglyceride to represent the amount of inflammation and/or oxidative stress. Therefore, in this study, we examined the health effect of lycopene on metabolic syndrome based on the ratio of serum lycopene to serum triglyceride.

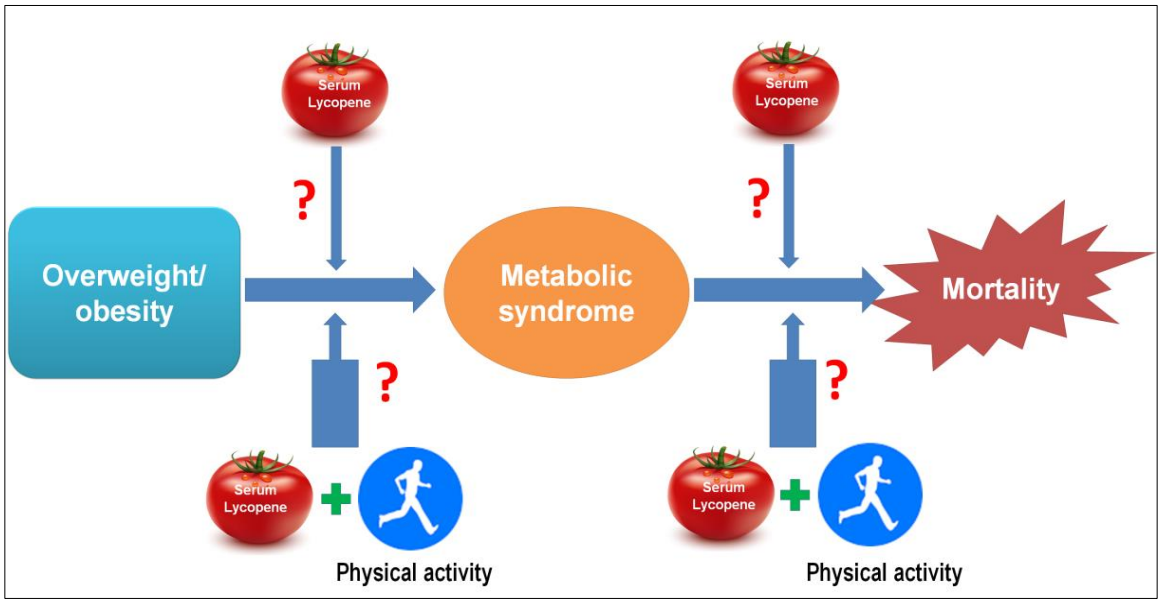
Metabolic syndrome also increases the risk of death from all causes as well as cardiovascular diseases [7, 8]. In addition to decreasing the risk of cardiovascular diseases, lycopene was also found to reduce the mortality in the general population [61, 62] and in patients with Alzheimer's disease or obstructive lung function [63, 64]. Therefore, serum lycopene also has a potential to reduce the risk of mortality for individuals with metabolic syndrome. However, some intervention studies did not find significant associations between lycopene and cardiovascular diseases [65, 66] and mortality [67]. Different criteria for the amount of increase in the serum concentration of lycopene may be an alternative explanation for these inconsistent results. For this reason, the ratio of the serum concentration of lycopene to the amount of inflammation and/or oxidative stress (relative serum concentration of lycopene) was used to predict the association between lycopene and mortality in this study.

As a healthy lifestyle, physical activity can reduce the risk of metabolic syndrome [43-45] and mortality [69]. Due to common biological mechanisms by which physical activity and serum lycopene regulate inflammatory cytokines [20, 21, 49-54], it is natural to posit that there will be additive effects between physical activity and serum lycopene on metabolic syndrome and mortality. Therefore, we examined the possible additive effects of physical activity and lycopene on metabolic syndrome and mortality in this study.

Figure 1 shows a conceptual model developed for this study. Overweight and obese individuals have a higher risk to develop metabolic syndrome than normal weight individuals. Individuals with metabolic syndrome have a higher risk of mortality than individuals without metabolic syndrome. An increased risk of metabolic syndrome occurs in overweight/obese individuals due to increased chronic inflammation and oxidative stress. Lycopene, as a natural antioxidant, can alleviate oxidative stress and inhibit inflammation. Therefore, we hypothesized that lycopene has the potential to reduce the risk of metabolic

syndrome among overweight and obese individuals. Metabolic syndrome also increases the risk of death from all causes as well as cardiovascular diseases. In addition to decreasing the risk of cardiovascular diseases, lycopene was also found to reduce the mortality in the general population and patients with Alzheimer's disease or obstructive lung function. Therefore, we also hypothesized that lycopene has the potential to reduce the risk of mortality for individuals with metabolic syndrome. In addition, due to common biological mechanisms by which physical activity and serum lycopene regulate inflammatory cytokines, we hypothesized that there were additive effects of physical activity and lycopene on metabolic syndrome and mortality.

Figure 1. Conceptual model of metabolic syndrome



1.9. Scope of the Study

The study took advantage of the NHANES database, a unique population-based survey and laboratory measruments, which collects information about the health and nutrition intake of adults and children [70]. The NHANES sample represents the United States non-institutionalized civilian population residing in the 50 states and the District of Columbia.

The data collection in NHANES included a home interview and a follow-up physical examination. The CDC institutional review board approved the survey; in addition, before participants took part in the study, they all signed written informed consent forms. In this study, only adult participants who were at least 20 years of age were included. The combination of NHANES 2001–2006 included 15,431 adult participants (7,341 men and 8,090 women).

1.10. Summary

In this chapter, we first described the background of the research problems. Metabolic syndrome is an important health condition because it is directly related to a high risk of chronic diseases and mortality. The growing prevalence of metabolic syndrome is related to the increasing prevalence of overweight and obesity. Therefore, exploring healthy lifestyles, such as diet and physical activity, is very important to reduce the prevalence and mortality of metabolic syndrome for overweight and obese individuals. We stated our research purposes, specific aims/research hypotheses and theoretical framework. Lastly, we described the population source for this study. The data in this study was obtained from the NHANES from 2001 to 2006. The study is important because the findings can provide an important analysis method (the ratio of serum lycopene to serum triglyceride) for future studies between lycopene and health effects.

CHAPTER 2 REVIEW OF THE LITERATURE

2.1. Overweight and Obesity

Overweight and obesity are referred to be a body weight that's greater than what is considered healthy for a certain height [71]. BMI, an expression with weight divided by height squared (kg/m^2), is a common measure for classification of overweight and obesity because it is inexpensive and easy to use for clinicians and for the general public. An adult with a BMI between 25.0 and 29.9 kg/m^2 is considered to be overweight and an adult with a BMI of 30 kg/m^2 or higher is considered to be obese. For children and adolescents, when a BMI number is plotted on a specific growth chart, we can find a relative position for this BMI (specific gender-age-percentile). If his/her BMI is located between 85^{th} and 95^{th} percentile, he/she is considered as overweight; if his/her BMI is located equal or greater than 95^{th} percentile, he/she is considered as obese [72].

According to the Global Burden of Disease Study, approximately 35% adults aged 20 years and above were overweight or obese in 2008 around the world [73]. Obesity epidemic has become a major public health problem in the United States. The prevalence of obese adults had more than doubled from 15% in 1976-1980 to 36% in 2009-2010 in the country [74]. The prevalence of adults who are overweight or obese was 68% for the period 2011-2012 [75]. This means two-thirds of American adults are either overweight or obese. According to Allison et al., 280,000 to 325,000 deaths per year are attributable to overweight and obesity and their complications in the United States. [76].

Overweight and obesity place a dramatic burden on families and society. There are many methods for economic estimation; the common methods include the direct and indirect cost estimations [77]. Direct costs include physician visit and hospital or nursing home care. Indirect costs include the income loss resulted from work loss, and premature death.

In 1995, the total estimated costs related to overweight and obesity were approximately $99.2 billion, including $51.6 billion in direct costs and $47.6 billion in indirect costs [77]. By 2000, the total costs of overweight and obesity were $117 billion, with $61 billion in direct costs and $56 billion in indirect costs [78]. For youths aged 6 to 17 years, overweight and obesity-related hospital costs had increased three times from $35 million (1979-1981) to $127 million (1997-1999) per year [79]. Overweight and obesity had led to over 1.3 billion long-term care patient days and over $68 billion Medicaid costs (in 2012 value) among baby boomers [80].

2.2. Roles of Excess Weight and Metabolic Syndrome in Development of Chronic Diseases

These significant public health burdens related to overweight and obesity mainly contribute to its high risk of developing chronic diseases, such as cardiovascular disease [81, 82], cerebrovascular disease [83] diabetes [84], osteoarthritis [85], non-alcoholic fatty liver disease [86] and cancers [87]. Cardiovascular disease, cerebrovascular disease and diabetes are some of the leading causes of mortality in the world [88]. Accumulating epidemiological evidence supports that a cluster of metabolic disorders in overweight and obese individuals is related to high risks of developing cardiovascular and cerebrovascular diseases. The cluster of metabolic disorders includes insulin resistance or higher fasting glucose, higher blood pressures, higher plasma triglyceride, and lower HDL cholesterol concentrations. For example, the growing prevalence of insulin resistance is significantly related to increasing BMI [89]. The prevalence of hypertension is higher in obese (35.7%) and overweight (26.4%) than in normal weight adults (19.8%). And the prevalence of dyslipidemia is higher in obese (49.7%) and overweight (44.2%) than in normal weight adults (28.6%) [90]. This cluster of metabolic disorders was named as insulin-resistance

syndrome (syndrome X) at first [91, 92], then was named as metabolic syndrome lately [93].

2.3. Metabolic Syndrome

According to the American Heart Association and the National Heart, Lung, and Blood Institute, metabolic syndrome is defined as a condition in which a group of metabolic risk factors exist in an individual. These risk factors, such as obesity, dyslipidemia, elevated blood pressure, insulin resistance, are directly related to a high risk of cardiovascular disease and type 2 diabetes mellitus [1].

Currently, there are at least three clinical diagnosis criteria recommended for metabolic syndrome. According to the World Health Organization (WHO), if an individual has glucose intolerance, impaired glucose tolerance (IGT) or diabetes mellitus (DM) and /or insulin resistance together with two or more of the following components: raised arterial pressure (greater and equal to 140/90 mmHg); dyslipidemia [elevated plasma triglycerides (greater and equal to 1.7mmol/L) and/or decreased HDL-cholesterol (less than 0.9 mmol/L for men and less than 1.0 mmol/L for women)]; central obesity (waist to hip ratio great than 0.90 for men and great than 0.85 for women) and/or BMI greater than 30 kg/m^2; and microalbuminuria (urinary albumin excretion rate greater and equal to 20 ug/min or albumin to creatinine ratio greater and equal to 30 mg/g) [94]. The American Association of Clinical Endocrinologists proposes another set of clinical criteria: overweight/obesity (BMI greater and equal to 25 kg/m^2); elevated triglycerides (greater and equal to 1.69 mmol/L); low HDL-cholesterol (less than 1.04 mmol/L for men and less than 1.29 mmol/L for women); elevated blood pressure (greater and equal 130/85 mmHg); two hours post-glucose challenge (greater than 140 mg/dl); fasting glucose (between 110 mg/dl and 126 mg/dl) and other risk factors (such as family history of T2DM, hypertension, or CVD; sedentary lifestyle; advancing age and high risk ethnic groups) [95]. According to the National

Cholesterol Education Program's Adult Treatment Panel III report (ATP III), if an individual has three or more of the following criteria, he/she will be diagnosed with metabolic syndrome: abdominal obesity (waist circumference greater than 102 cm for men and greater than 88 cm for women); hypertriglyceridemia (greater and equal to 1.69 mmol/L); low HDL-cholesterol (less than 1.04 mmol/L for men and less than 1.29 mmol/L for women); hypertension (greater and equal to 130/85 mmHg) and high fasting glucose (greater and equal to 6.1 mmol/L) [96].

Using the ATP III criteria, Ford et al. found the prevalence of metabolic syndrome among the United States adults was 24.0%. The study was based on the third National Health and Nutrition Examination Survey (NHANES III) from 1988 to 1994 [97].

Due to increased prevalence of overweight and obesity in the United States, the prevalence of metabolic syndrome continues to increase. Using the ATP III criteria, Aguilar et al. found the prevalence of metabolic syndrome among United States adults to be 32.9% during 2003-2004 and 34.7% during 2011-2012 based on NHANES 2003-2012 [98].

2.4. Inflammation, Oxidative Stress, Obesity and Metabolic Syndrome

Why is the percent of metabolic syndrome higher in individuals who are overweight and obese than individuals who have normal weight? Inflammation and oxidative stress are the central mechanisms connecting overweight and obesity to metabolic syndrome [11, 12, 99, 100]. Studies show that adipose tissue is one of the sources of inflammation and oxidative stress [101-105]. For example, many inflammatory cytokines, such as TNF-α, IL-1β, IL-6, IL-8, IL-10 and leptin can produce in adipose tissue [101-103]. In addition to cytokines, adipose tissue can produce lipid peroxides and oxidative stress, such as reactive oxidative metabolites [104, 105]. Therefore, excess adipose tissue can lead to more inflammation and oxidative stress in individuals who are overweight and obese. For

example, there are significantly increased oxidative stress (oxidized low-density lipoprotein and reactive oxygen species) and low-grade inflammation (C-reactive protein and leptin) in obese individuals when compared to normal weight individuals [13, 106]. In addition, antioxidant enzymes and total plasma antioxidant capacity are suppressed in overweight and obese condition. For example, dietary-induced obesity can decrease antioxidant enzymes, such as superoxide dismutase (SOD), glutathione peroxidase in adult rats [107]. Furthermore, adipose glutathione peroxidase was greatly suppressed by prooxidative conditions such as high levels of TNF-α and hypoxia [108].

2.5. Prevention and Treatment of Metabolic Syndrome

Prevention of metabolic syndrome is needed to address several risk factors and reduce inflammation and oxidative stress in the body while treatments of metabolic syndrome are needed to control the components of metabolic syndrome.

Although metabolic syndrome can be caused by genetic factors, aging, smoking, unhealthy diets and inactive lifestyles [98, 109 and 110], overweight and obesity is the primary reason for metabolic syndrome [111]. Therefore, in addition to quitting smoking and keeping an active lifestyle, controlling weight is very important for the prevention of metabolic syndrome. Preventing overweight and obesity is an important step to reduce the prevalence of metabolic syndrome and chronic disease conditions and reduce the financial burden on patients' families and society. However, although some interventions related to overweight and obesity have been developed to improve the health of individuals and populations in recent years [112-114], the prevalence of overweight and obesity in the United States is still a huge problem [75].

Overweight and obesity are associated with increased oxidative stress and chronic inflammation status that favors the development of metabolic syndrome. Therefore, alleviating oxidative stress and/or suppressing inflammation is an alternative strategy to reduce the prevalence of metabolic syndrome among individuals who are overweight and obese. One is the therapeutic drug strategy. For example, salsalate, a traditional anti-inflammatory medication, can prevent metabolic syndrome in obese non-diabetic individuals [14]. Probucol, a potent anti-oxidant drug, has been in clinical use during the past few decades for the prevention of cardiovascular diseases [15]. However, these therapeutic drug strategies that target weight-related oxidative stress and/or inflammation can potentially reduce chronic disease burden, but often anti-oxidants and anti-inflammatory drugs have serious side effects in long-term disease prevention, such as approximately 30% of patients discontinued the use of salsalate because of gastrointestinal symptoms and tinnitus [16] and many patients discontinued the use of probucol because of QT interval prolongation [17].

A diet rich in antioxidants is another potential approach to reduce oxidation and inflammation. Carotenoids are a group of antioxidants that are found in fruits and vegetables. There are over 600 different carotenoids identified, however, approximately 90% of carotenoids are represented by the common dietary carotenoids including α-carotene, β-carotene, lycopene, lutein and β-cryptoxanthin [115]. A-carotene, β-carotene, and β-cryptoxanthin can convert to retinal and have vitamin A activity while lycopene and lutein cannot convert to retinal and therefore have no vitamin A activity [116]. After absorption, carotenoids are mainly distributed in the adipose tissue [117, 118]. B-carotene and lycopene are the predominant components of carotenoids in the adipose tissue, approximately 20% of total carotenoids respectively [118]. Due to its higher tissue concentration and vitamin A activity, β-carotene is regarded as the main research interest in epidemiological

studies [119-122]. However, β-carotene may play double roles since β-carotene can promote health when taken at a lower level while β-carotene can bring adverse effects when taken at a higher level [115,120 and 121]. In addition, lycopene is discovered to be the most potent antioxidant; therefore, lycopene has attracted the attention of scientists around the world [123, 124].

2.6. Lycopene

As a natural antioxidant, lycopene can alleviate oxidative stress induced in animal models [125, 126]. Dietary intake of tomatoes or purified lycopene supplementation has also decreased the oxidative damages in humans [18, 19, 127 and 128]. For example, lycopene supplementation can reduce 86% of induced lipid peroxidation in monkey cells [125]. In humans, consumption of tomato products can reduce lipoprotein sensitivity to oxidative damage [18] and reduce biomarkers of oxidative stress, such as lipid peroxidation and DNA damage [19, 127 and 128].

Lycopene is a red pigment found primarily in tomatoes. Structurally, lycopene, assembled from eight isoprene units, is a tetraterpene that is not soluble in water. Like all other carotenoids, lycopene is a polyunsaturated hydrocarbon. Lycopene's antioxidant activity attributes to its eleven conjugated double bonds [129]. The main source of lycopene for humans is from dishes prepared with vegetables and fruits which contain lycopene. Lycopene is mainly contained in tomatoes and other vegetables or fruits, such as red carrots and watermelons. There is about 3,014 µg lycopene per 100g tomatoes; 1µg per 100g carrots and 4,532 µg per 100g watermelon [130].

In general, there are two kinds of lycopene, trans-lycopene and cis-lycopene. Trans-lycopene is its natural form that is long and straight while cis-lycopene is a bent form that circulates in human blood. Trans-lycopene comprises 79-91% of total lycopene in tomato

and tomato products, while cis-lycopene makes up more than 50% of the total lycopene in human plasma [131, 132]. Isomerization is a process that converts trans-isomers to cis-isomers. Although the majority of isomerization is reported to occur in vivo, the mechanism of converting trans-lycopene to cis-lycopene in serum and tissues is not clear [133]. Trans-lycopene and cis-lycopene have similar chemical properties. There is no significant difference in antioxidant activity between trans-lycopene and cis-lycopene [134].

2.7. Mechanism of Lycopene

After absorption from the intestine, lycopene is primarily distributed in adipose tissue (60-72%), liver (17-23%), and serum or plasma (5-6%) [135]. Although the mechanism of different distribution of lycopene is unclear, the distribution varying in different tissue may suggest different functions for different tissues [135]. Lycopene concentrations at different adipose tissue sites (abdomen, buttock, and thigh) have positive correlations with serum lycopene concentration [136]. As a natural antioxidant, lycopene can alleviate oxidative stress induced in animal models [125, 126]. Dietary intake of tomatoes or purified lycopene supplementation has also decreased the oxidative damages in humans [18, 19, 127 and 128]. In addition, animal experiments demonstrate that lycopene can significantly decrease pro-inflammatory cytokine and chemokine expression by inhibiting the TNFα-mediated activation of the NF-κB signaling pathway in vitro and in vivo [20, 21]. Therefore, alleviating oxidative stress and suppressing inflammation may be the main mechanisms for the effects of lycopene [137]. Serum concentrations of lycopene were measured using high-performance liquid chromatography with multi-wavelength photodiode-array absorbance detection [138].

Lycopene was found to reduce the risks of developing chronic diseases. For example, since the first report that lycopene can reduce the incidence rate of prostate cancer in

1995 [26], lycopene has been found to be inversely associated with breast, cervical, ovarian, liver and other organ site cancers [27]. In addition to tumor-suppressive activity, lycopene is found to reduce the risks of developing other chronic diseases, such as cardiovascular diseases and cerebrovascular diseases. These studies can usually be divided into dietary intake lycopene studies and serum lycopene studies. In general, studies based on lycopene intake failed to detect a significant association between lycopene and cardiovascular diseases and cerebrovascular diseases [139-141]. Misclassification of dietary lycopene intake may be an alternative explanation for the failure of these dietary studies [142].

There were many studies which examined the associations between lycopene and heart diseases. Kohlmeier and colleagues found that there was a dose-response inverse association between lycopene and myocardial infraction with a population-based case-control study in Europe (EURAMIC Study) [28]. Karppi and colleagues also found that low serum lycopene increased the risk of myocardial infarction in Finnish men [29]. However, Hak and colleagues did not find an association between serum lycopene and the risk of myocardial infarction in United States male physicians [65]. For coronary heart disease in Czech, Bavarian and Israeli men, Bobak and colleagues found there was a significant association between lycopene and coronary heart disease [30]. A cohort study (the Kuopio Ischaemic Heart Disease Risk Factor Study) found that the lower level of serum lycopene was associated with increased risks of coronary heart disease with 6 years of follow-up for Finnish people [31].

For stroke, Rissanen and colleagues found low serum levels of lycopene was associated with an increased risk of stroke with 6 years of follow-up for Finnish people [31]. With the same cohort data, Karppi and colleagues also found low serum levels of lycopene continued to be associated with an increased risk of stroke with 12 years of follow-up [143].

For the combination of disease conditions, Sesso and colleagues found that high levels of plasma lycopene concentration can reduce the incidence rate of cardiovascular disease in women [144]. However, Sesso and colleagues did not find that high plasma lycopene concentration was associated with the risk of cardiovascular disease in older men [66].

In addition to reducing the risk of cardiovascular disease and cerebrovascular disease, lycopene was found to improve endothelial function in participants with cardiovascular disease [145]. Kristenson and colleagues found that the mortality from coronary heart disease in Lithuania was associated with serum concentration of lycopene [146]. Ito and colleagues also reported that lycopene played an important role in preventing death from cardiovascular diseases in Japanese patients [61]. However, Karppi and colleagues did not find an association between the risk of cardiovascular disease mortality and serum concentration of lycopene among Finnish men [67].

For all-cause mortality, with NHANES III data, Patel and colleagues found that serum concentration of lycopene was negatively related to all-cause mortality [62]. However, with the same data, Shardell and colleagues only found the middle two quartiles had an association with all-cause mortality while the lowest quartile and the highest quartile had no association with all-cause mortality [147]. Furthermore, the authors found high serum concentration of lycopene up to 0.40 μmol/L was associated with a reduced mortality. Starting at 0.50 μmol/L, a high serum concentration of lycopene was associated with increased mortality ($P<0.001$). Therefore, the authors indicated that there may be a U-shaped relationship between serum lycopene and mortality [147].

2.8. Lycopene Measurement and Factors Associated with Lower Serum Lycopene

Usually, there are two methods for lycopene measurement: serum concentration of lycopene and dietary intake of lycopene. Although most lycopene is distributed in adipose

tissue [135], it is not common to test lycopene concentration in adipose tissue because it is inconvenient to detect in these tissues. In addition, serum lycopene concentration has a positive correlation with lycopene concentration in adipose tissue [136]. Therefore, serum or plasma lycopene is the common measure for humans. Lycopene concentration can be measured in serum by reverse-phase high-performance liquid chromatography [33] while dietary intake of lycopene can be estimated by using 24-hour food recalls (values were converted to mg per day based on checklist of tomato-based foods) [34]. Serum concentration of lycopene is an objective test measurement while dietary intake of lycopene is an subjective measurement of self-reported data. Therefore, serum concentration of lycopene is more valid and reliable than dietary intake of lycopene [142].

Studies show that there are several factors related to high (or low) levels of serum lycopene. For example, race/ethnicity, gender and age have associations with serum concentration of lycopene [148-150]. In addition, lifestyles can impact serum concentration of lycopene. For example, cigarette smoking was inversely associated with serum concentration of lycopene [151]. The effects of alcohol consumption on serum concentration of lycopene are inconsistent in previous reports [152, 153].

2.9. Lycopene Levels among Overweight and Obese Individuals

Individuals who are overweight or obese tend to have less healthy dietary habits. For example, usually, overweight or obese individuals consume too much energy and consume much less fruits and vegetables [154, 155]. Lycopene is mainly contained in tomatoes and other vegetables or fruits, such as red carrots and watermelons. These findings suggest that serum lycopene levels are lower among overweight and obese individuals than normal weight individuals. However, there is not sufficient evidence to support this notion. Two small sample studies found a lower level of lycopene among obese individuals compared to normal weight individuals but the difference was not statistically

significant [36, 156]. To overcome the small sample size, the data based on the third NHANES 1988-1994 (N=3,413) was used for studying the association between serum concentration of lycopene and BMI status. The authors did not report results for serum concentration of lycopene among normal weight, overweight and obese individuals but they mentioned that BMI was not associated with serum lycopene in an multivariate adjusted regression analysis of serum lycopene concentration [149].

With the same dataset, Kimmons and colleagues found that the low serum lycopene levels (20th percentile by age and gender as the cutoff) were inversely associated with BMI for women and not for men [157]. Therefore, these inconsistent results may also contribute to the difference criteria for the definition of “high” and “low” serum concentration of lycopene because no standard criteria exist for serum concentration of lycopene in the body. Therefore, relative serum concentration of lycopene to the amount of oxidative stress and/or inflammatory reaction is more rational for the definition of “high” or “low” serum concentration of lycopene according to self-requirement in the body.

2.10. The Health Effects of Lycopene in Overweight and Obese Adults

Overweight and obese individuals have an increased risk of developing metabolic syndrome due to subsequent chronic inflammation and oxidative stress [11, 12, 99 and 100]. Alleviating oxidative stress and suppressing inflammation are the main mechanisms for the effects of lycopene [137]. Therefore, lycopene has the potential to reduce the risk of metabolic syndrome by suppressing inflammation and oxidative stress in overweight and obese individuals.

The health effects of lycopene on reducing inflammation and oxidative stress remain inconsistent in overweight and obese adults. For example, Yeon et al., McEneny et al. and Ghavipour et al. found that lycopene intervention can significantly reduce inflammation

and oxidative stress in overweight and obesity participants [32-34], while Markovits et al. and Thies et al. did not find a significant association between inflammation and oxidative stress and lycopene [36, 37]. Therefore, health effects of lycopene for individuals who are overweight and obese are still controversial.

One of the potential reasons for the varying effects of lycopene is differences in the amount of serum lycopene in these studies. For example, in participants who were of similar race, age range, and BMI status, greatly increased concentrations of lycopene (1140 umol/L) significantly affected inflammation and/or oxidative stress [33], while a small increase in the cncentration of lycopene (7.61 umol/L) did not have significant effects [37]. Therefore, health effects of serum lycopene may also depend on the serum concentration of lycopene.

Another point to consider when interpreting the research findings on lycopene is variations in the levels of oxidation and inflammation in different individuals. For example, based on equal serum concentrations of lycopene, it is possible that only in participants who have a lower BMI, lycopene can effectively reduce the levels of TNF-α and IL-8 and increase the levels of superoxide dismutase, glutathione peroxidase and catalase, and total antioxidant capacity of plasma [34, 35]. The same “high” serum concentrations of lycopene for individuals with a lower BMI status do not produce significant health effects for individuals with a higher BMI status due to increased inflammation and oxidative stress production [34, 35]. Therefore, health effects of serum lycopene appear to depend on the amount of inflammation and/or oxidative stress in the body.

Taken together, health effects of serum lycopene depend not only on serum concentration of lycopene, but also on the amount of inflammation and/or oxidative stress in the body. Therefore, we hypothesized that the ratio of serum concentration of lycopene to the

amount of inflammation and/or oxidative stress could better predict health effects of serum lycopene than with only the serum concentration of lycopene.

2.11. Summary

In this chapter, we first reviewed overweight/obesity, inflammation/oxidative stress and metabolic syndrome. Then we reviewed nutrient intake for prevention of metabolic syndrome, especially for lycopene. Accumulative evidence supports that lycopene can reduce the risk of metabolic syndrome and chronic disease. Alleviating oxidative stress and suppressing inflammation are the main mechanisms for the effects of lycopene. However, the health effects of lycopene on reducing inflammation and oxidative stress remain inconsistent in overweight and obese adults. These inconsistent results may be due to different serum concentrations of lycopene or different amounts of inflammation and/or oxidative stress in the participants' body. We hypothesized that the ratio of serum lycopene to the amount of inflammation and/or oxidative stress may be a better measure for analysis of health effect of lycopene than with only the serum concentration of lycopene.

CHAPTER 3 RESEARCH METHODS

3.1. Overview

This is a secondary analysis of data from a population-based cross-sectional survey conducted in the United States. This study used publicly available NHANES data. The NHANES 2001–2006 data included 15,431 participants who were at least 20 years old (7,341 men and 8,090 women). The outcome variables were metabolic syndrome and mortality. The exposure variables included serum concentration of lycopene, the ratio of serum lycopene to serum triglyceride and physical activity. Chi-square tests, multivariate logistic regressions and Cox models were used in association analyses.

3.2. NHANES

The NHANES is conducted by the National Center for Health Statistics of the CDC to assess the health and nutrition conditions of adults and children in the United States. We used the 2001-2006 NHANES data. The study samples included all participants aged 20 years and older. There were 5,411 adult participants in 2001-2002, 5,041 in 2003-2004 and 4,979 in 2005-2006. Individuals were excluded if they had missing information for classification of metabolic syndrome and serum concentration of lycopene. For an analysis of the additive effect of physical activity and lycopene, individuals were excluded if they had missing information for physical activity. For a mortality analysis, individuals were excluded if they had diabetes, heart diseases and stroke history or if their underlying causes of death were from these conditions. Using a multistage, stratified sampling design, the NHANES obtains a representative sample of civilian, non-institutionalized individuals in the United States. In the first sample stage, researchers selected the primary sampling units (PSUs) from the sampling frame including all counties in the United States with PPS (probabilities proportionate to the measure of size) sample technique. PPS means that the

likelihood of being selected is proportionate to the size of the unit. In the second sample stage, researchers selected the segments from the sample frame including all census blocks or combined blocks in the selected PSUs in the first sample stage with PPS technique. In the third sample stage, researchers selected dwelling units (DUs) from the sample frame including all non-institutional group quarters in the selected segments in the second sample stage. To obtain reliable estimates of health and nutritional measures, oversampling for some subgroup populations were designed in this stage. For example, non-Hispanic African Americans and Mexican Americans were oversampled during 2001-2006. In the fourth sample stage, researchers selected individuals from the list within DUs or households based on a previously defined probability for different race, income, gender and age groups [138, 158].

Data collections include home interviews and examinations in NHANES survey. The NHANES home interviews included demographic, socioeconomic, dietary, and health-related questions. A trained household interviewer administered the questionnaires in the sample participant's home. The interview was conducted using a computer-assisted personal interview system and Blaise software [138, 158]. The NHANES examinations took place in the mobile examination center (MEC). The examination component consists of medical, dental, and physiological measurements, as well as laboratory tests administered by highly trained medical personnel. Eligibility for examination components was determined by the participant's age and gender. The controlled environment of the MEC allowed physical measurements to be done under identical conditions at each survey location [138, 158].

3.3. Mortality Follow-Up

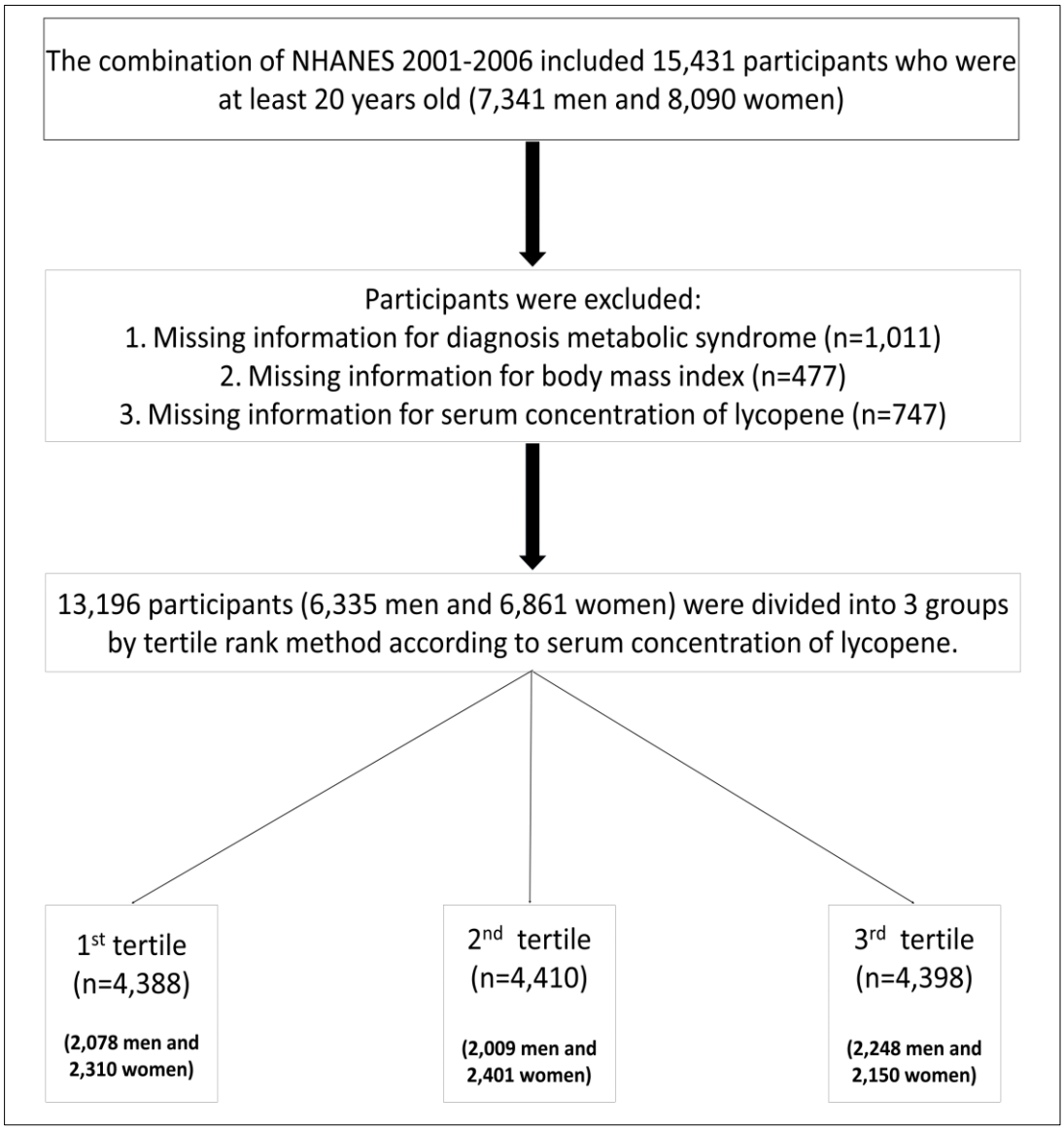
The NHANES 2001-2006 Linked Mortality File provided mortality information for participants involved in NHANES interview (2001-2006) through December 31, 2011. The

method of probabilistic linkage was used to trace NHANES participants with the National Death Index (NDI) for vital status. The linkage between survey participants and NDI can provide important information to conduct outcome studies, for example, in this study, the association between serum lycopene and mortality and the possible additive effect of physical activity and lycopene on mortality.

3.4. Study Samples

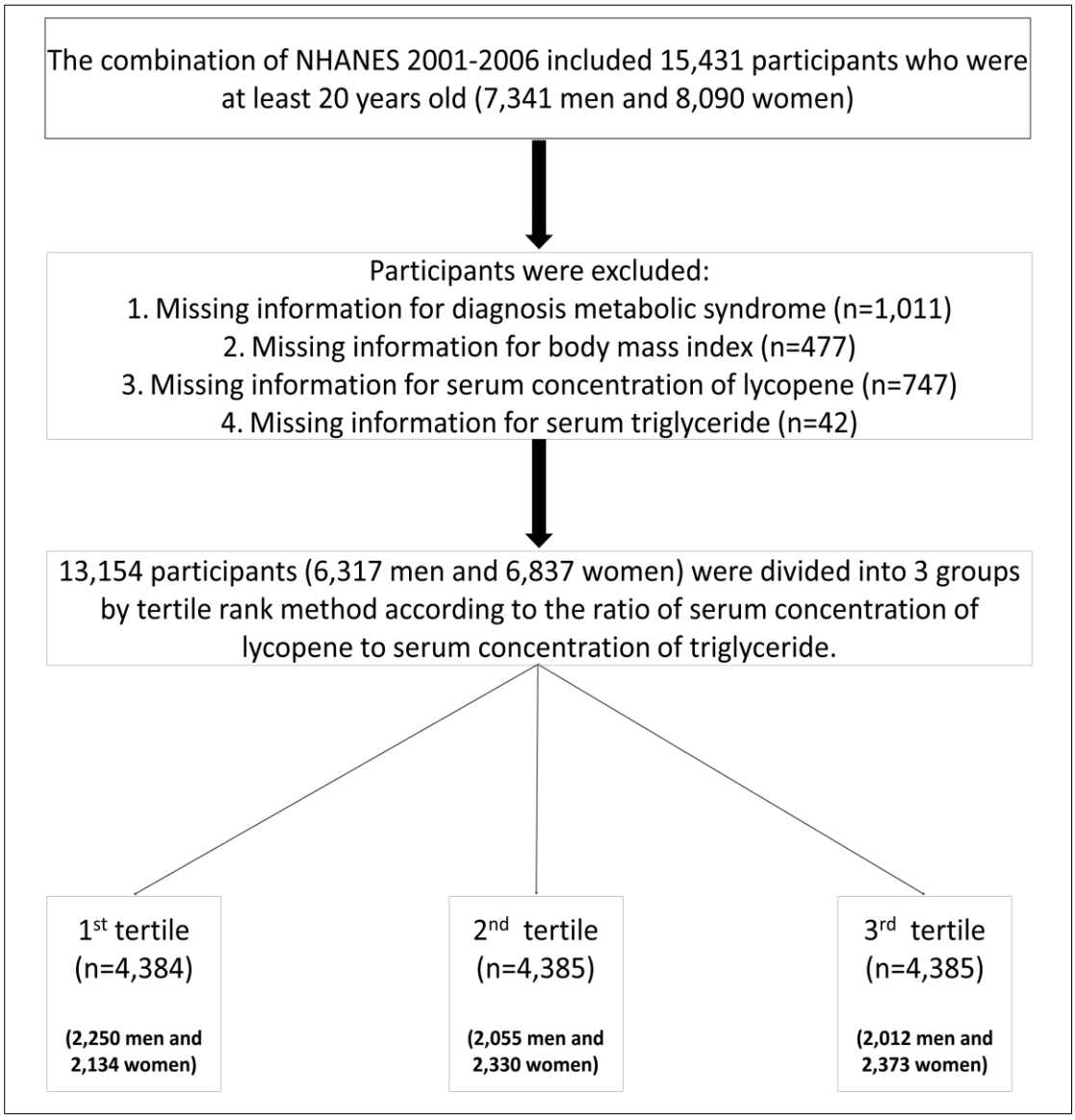
The sample for Aim 1 consisted of a total of 13,196 participants from the 2001-2006 NHANES. Figure 2 shows how this sample was derived and categorized into three groups according to the serum lycopene concentration. Of the 15,431 participants of the NHANES 2001-2006, a total of 2,235 were excluded due to missing information on metabolic syndrome (n=1,011), BMI (n=477), and serum lycopene concentration (n=747).

Figure 2. Aim 1 sample flow chart



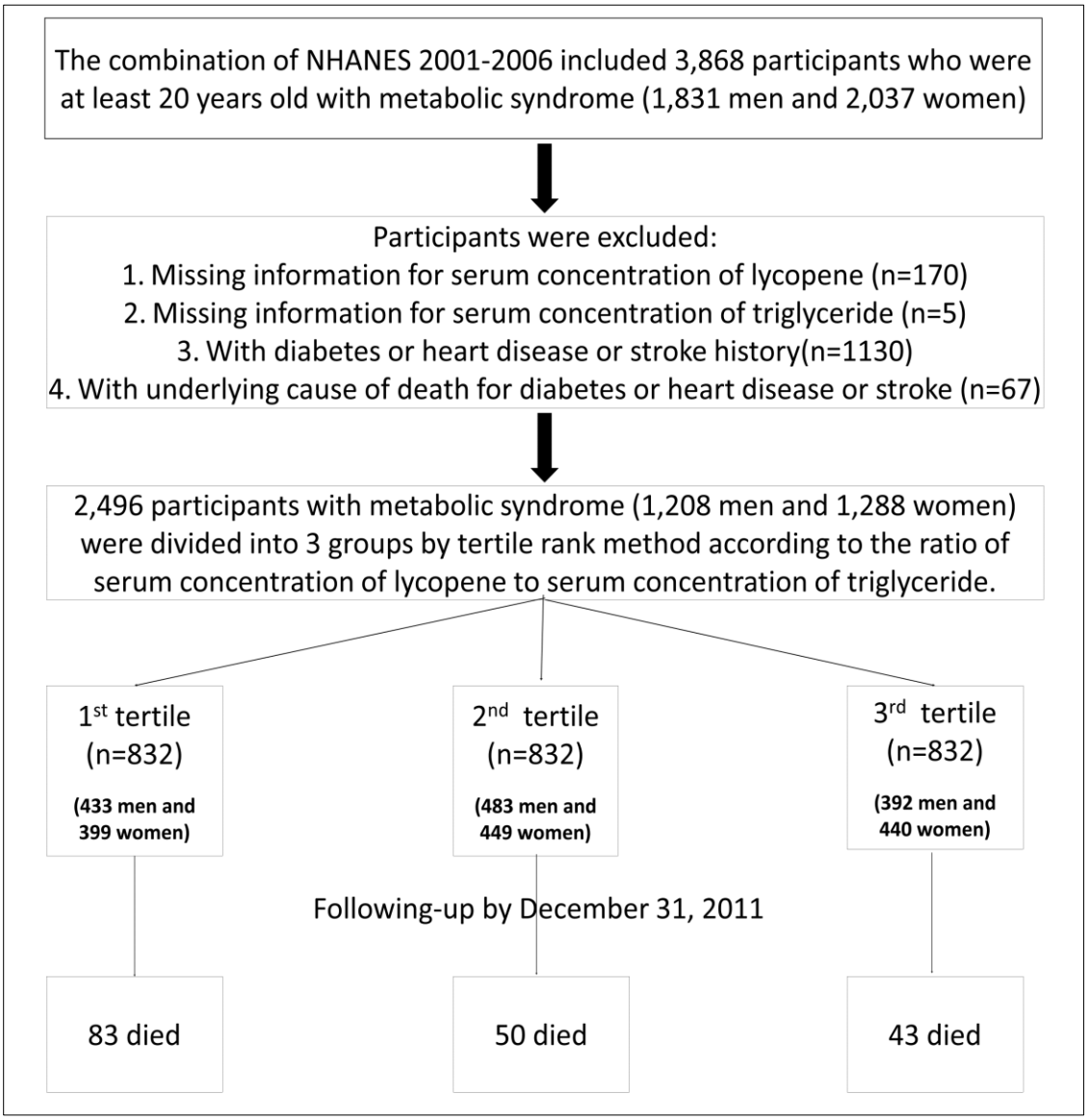
The sample for Aim 2 consisted of a total of 13,154 participants from the 2001-2006 NHANES. Figure 3 shows how this sample was derived and categorized into three groups according to the ratio of serum concentration of lycopene to serum concentration of triglyceride. Of the 15,431 participants of the NHANES 2001-2006, a total of 2,277 were excluded due to missing information on metabolic syndrome (n=1,011), BMI (n=477), serum lycopene concentration (n=747), and serum triglyceride concentration (n=42).

Figure 3. Aim 2 sample flow chart



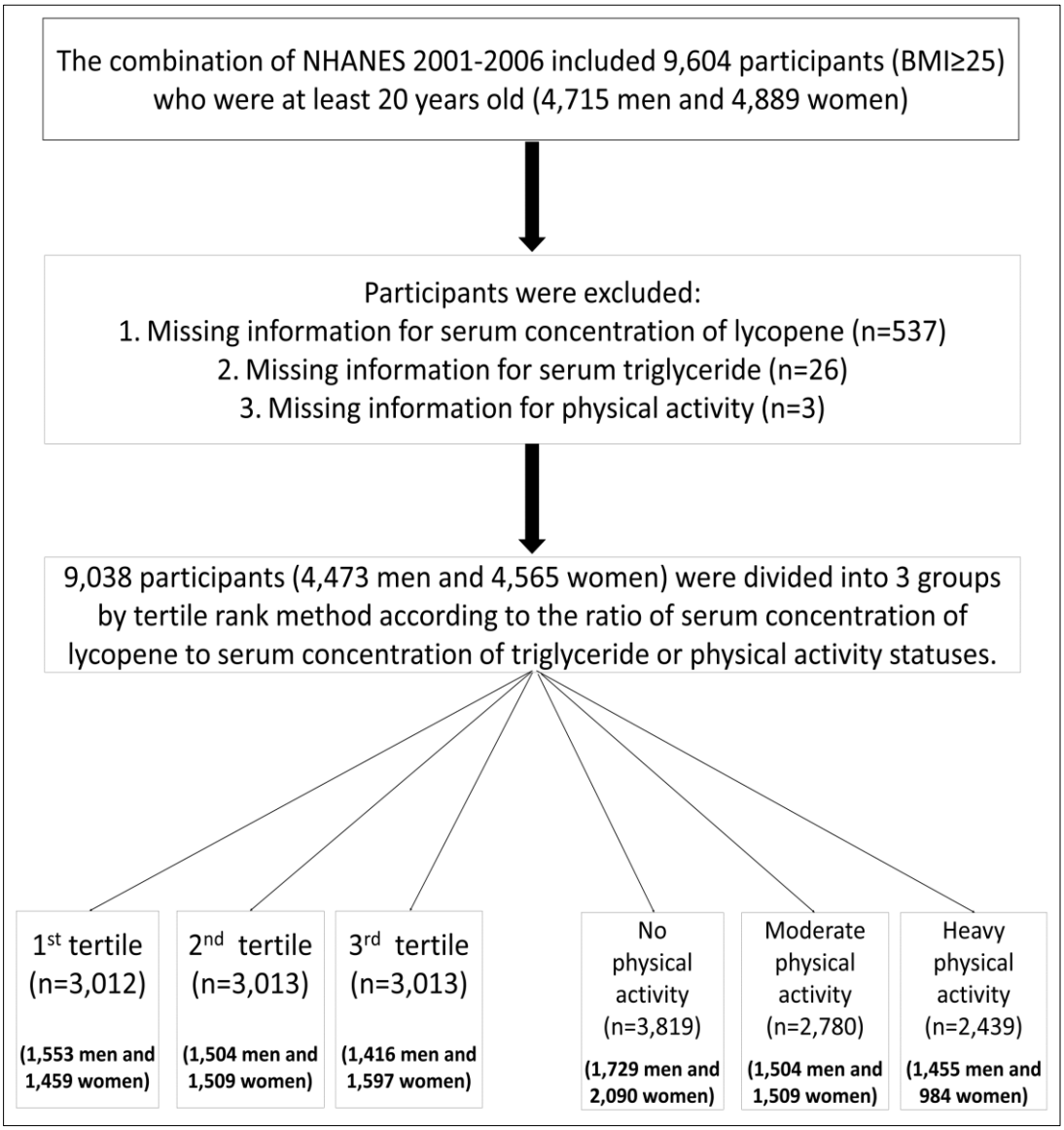
The sample for Aim 3 consisted of 2,496 participants from the 2001-2006 NHANES (Figure 4). Of the 3,868 participants with metabolic syndrome of the NHANES 2001-2006, a total of 1,372 were excluded due to missing information on serum lycopene concentration (n=170) and serum triglyceride concentration (n=5) or due to a history of diabetes or heart disease or stroke (n=1,130) or due to underlying causes of death for diabetes or heart disease or stroke (n=67).

Figure 4. Aim 3 sample flow chart



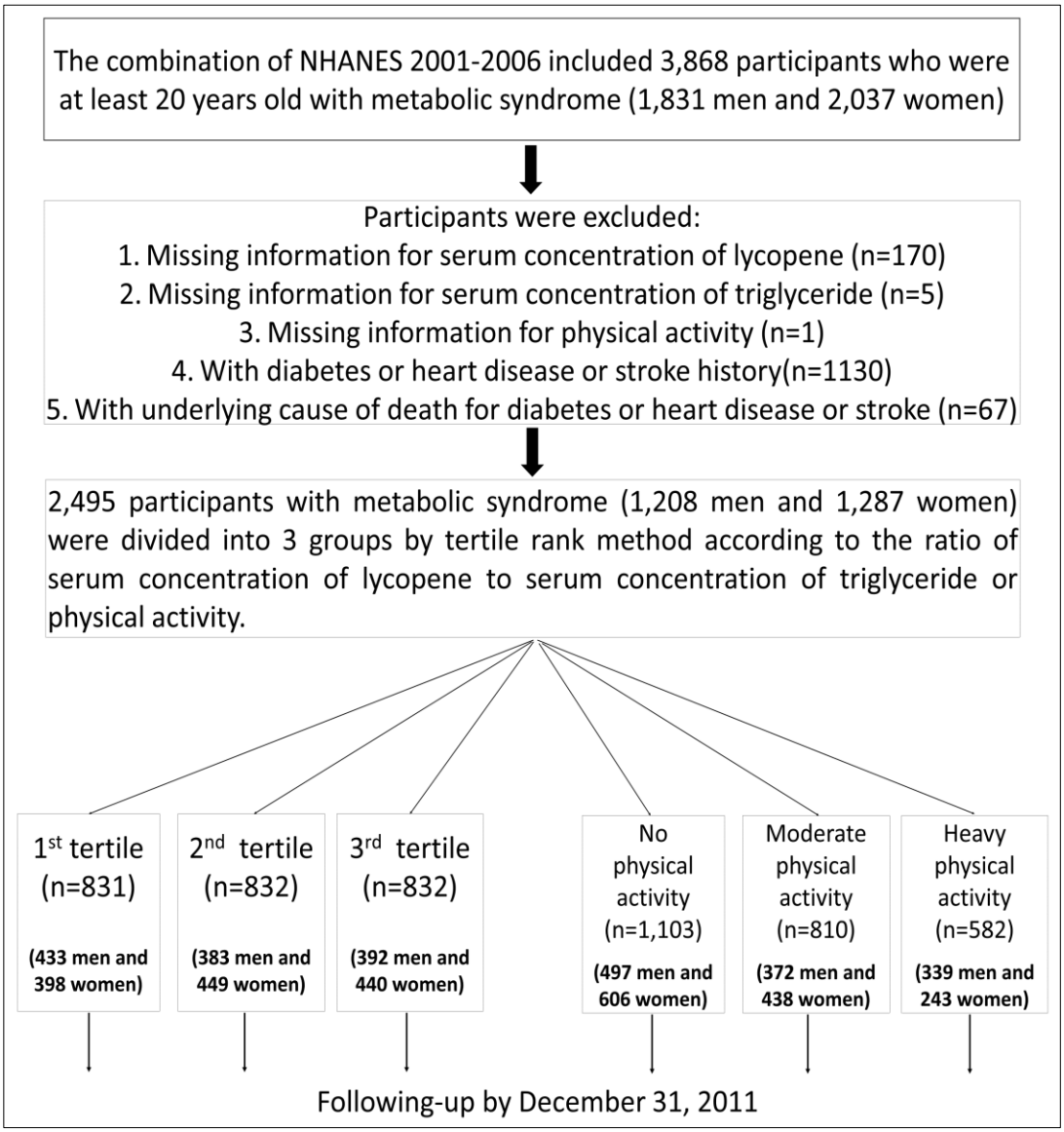
The sample for Aim 4 consisted of a total of 9,038 participants from the 2001-2006 NHANES. Figure 5 shows how this sample was derived and categorized into three groups according to the ratio of serum concentration of lycopene to serum concentration of triglyceride or physical activity statues. Of the 9,604 participants (BMI≥25) of the NHANES 2001-2006, a total of 566 were excluded due to missing information on serum lycopene concentration (n=537), serum triglyceride concentration (n=26), and physical activity (n=3).

Figure 5. Aim 4 sample flow chart



The sample for Aim 5 consisted of 2,495 participants from the 2001-2006 NHANES (Figure 6). Of the 3,868 participants with metabolic syndrome of the NHANES 2001-2006, a total of 1,373 were excluded due to missing information on serum lycopene concentration (n=170), serum triglyceride concentration (n=5) and physical activity (n=1) or due to history with diabetes or heart disease or stroke (n=1,130) or due to underlying causes of death for diabetes or heart disease or stroke (n=67).

Figure 6. Aim 5 sample flow chart



3.5. Measurements

3.5.1. Metabolic syndrome assessment and classification

The guideline provided in the National Cholesterol Education Program's Adult Treatment Panel III report was used to identify individuals with metabolic syndrome. To be diagnosed with metabolic syndrome, an individual has to meet three or more of the following criteria: abdominal obesity (waist circumference >40 inches for men and >35 inches for women); hypertriglyceridemia (serum triglyceride ≥1.69 mmol/L); low HDL-cholesterol (HDL <1.04 mmol/L for men and <1.29 for women); hypertension (≥130/85 mmHg) and high fasting glucose (fasting glucose ≥6.1 mmol/L) [2]. In this study, waist circumference (BMXWAIST), serum triglyceride (LBDSTRSI), serum HDL-cholesterol (LBDHDDSI), systolic blood pressure/ diastolic blood pressure and fasting glucose (LBDGLUSI) were used for classification of metabolic syndrome.

3.5.2. BMI assessment and classification

Overweight and obesity are conditions of excess fat accumulation in the body. BMI, an expression with weight divided by height squared (kg/m^2), is a common measure for classification of overweight and obesity. An adult with a BMI less than 24.9 kg/m^2 is considered to be normal weight; an adult with a BMI between 25.0 and 29.9 kg/m^2 is considered to be overweight and an adult with a BMI of 30 kg/m^2 or higher is considered to be obese.

3.5.3. Lycopene assessment and classification

Serum concentration of trans-lycopene was measured using high-performance liquid chromatography with multi-wavelength photodiode-array absorbance detection. Serum triglyceride was measured enzymatically by using a series of coupled reactions by Hitachi 717 analyzer [70].

For dietary measurement, NHANES collected two 24-hour dietary recalls for every participant by using the United States Department of Agriculture (USDA)'s Automated Multi-Pass Method. The first 24-hour recall was conducted at the MEC; the second 24-hour recall was conducted by telephone 3-10 days later. Four data files were created from dietary interview information: two individual foods files and two total nutrient intake files. The individual foods data files include information about the types and amounts of individual foods, as well as amounts of nutrients from each food. The nutrient amounts in the total nutrient intake files only reflect nutrients acquired from foods and beverages reported by participants. The nutrient amounts in the total nutrient intake files do not include nutrients from dietary supplements, medications, or plain drinking water. For example, total dietary lycopene only reflects intake from foods and beverages and not including any from dietary supplements and medications [138].

3.5.4. Physical activity assessment and classification

Physical activity was categorized into no physical activity, moderate physical activity and heavy physical activity. The categories of physical activity were defined based on the two questions: (1) "Over the past 30 days, did you do any vigorous activities for at least 10 minutes that caused heavy sweating, or large increases in breathing or heart rate?" and (2) "Over the past 30 days, did you do moderate activities for at least 10 minutes that cause only light sweating or a slight to moderate increase in breathing or heart rate?" If

the answer to the first questions was “Yes”, participants were classified as “Heavy physical activity”. If the answer to the second question was “Yes” and the answer to the first question was anything other than “Yes”, participants were classified as “Moderate physical activity”. If the answers to both questions were “No” or “Unable to do activity”, participants were classified as “No physical activity”.

3.5.5. Alcohol consumption assessment and classification

Alcohol consumption values were categorized based on two questions: (1) “Have you had at least 12 alcohol drinks/1 year?” and (2) “In the past 12 months, on those days that you drank alcoholic beverages, on the average, how many drinks did you have per day?” If the answer to the first question was “No”, participants were classified as “No alcohol consumption”. The alcohol consumption level was classified as “Moderate alcohol consumption” if the participant answered “Yes” to the first question and “Average alcoholic drinks/per day less than or equate to 2 for men and less than or equate to 1 for women” to the second question; the alcohol consumption level was classified as “Heavy alcohol consumption” if the participant answered “Yes” to the first question and “Average alcoholic drinks/per day more than 2 for men and more than 1 for women” to the second question.

3.5.6. Smoking assessment and classification

Smoking status was categorized based on three questions: (1) “Have you smoked at least 100 cigarettes in your entire life?” (2) “Do you now smoke cigarettes?” and (3) “During the past 30 days, on the days that you smoked, about how many cigarettes did you smoke per day?” If the answer to the first question was “No”, participants were classified as “No smoker”; If the answer to the first question was “Yes” and the answer to the second question was “Not at all”, participants were classified as “Past smoker”. Smoking status was classified as “Current smoker” if the participant answered “Yes” to the first question

and “Every day or some days” to the second question and “Average more than 1 cigarette /per day” to the third question.

3.5.7. Assessment and classification of other variables of interest

In this study, demographic variables included race/ethnicity (Non-Hispanic White, Non-Hispanic Black, Mexican American and others), gender (Male and Female), and age group (20–39, 40–59 and ≥60 years). Common chronic disease conditions included diabetes, heart disease, stroke, cancer. Diabetes was defined as answering “Yes” to the question “Whether a doctor told you that you had diabetes?”; and heart disease was defined answering “Yes” to any of the following three questions “Has a doctor told you that you had coronary heart disease?” or “Has a doctor told you that you had angina/angina pectoris?” or “Has a doctor told you that you had heart attack?”; and stroke was defined if the participant answered “Yes” to the question “Has a doctor told you that you had stroke?” Cancer or malignancy was defined as answering “Yes” to the question “Has a doctor told you that you had cancer or a malignancy of any kind?”

3.6. Missing Data

The main purpose of this study was to examine the associations between serum lycopene and metabolic syndrome or mortality. Therefore, the accuracy was very important for this study. Imputing a value for the missing data, such as serum concentration of lycopene, could potentially introduce inaccurate values into the data. Therefore, we did not choose to impute a value for the missing data. We compared the sample characteristics between the original and the final samples. The percent of men was 47.6% in the original sample (15,431) and 48.0% in the final sample (13,196). The percent was 36.2% for participants aged 20-39 years, 29.2% for participants aged 40-59 years and 34.6% for participants aged 60 years and older in the original sample; the percent was 36.8% for

participants aged 20-39 years, 30.4% for participants aged 40-59 years and 33.8% for participants aged 60 years and older in the final sample. The prevalence of metabolic syndrome was 26.8% in the original sample and 27.2% in the final sample. Therefore, after excluding the participants with missing information, the sample characteristics did not change.

3.7. Statistical Analyses

3.7.1. Survey weights

Statistical analyses were performed using SAS Survey Procedures (i.e., proc surveyfreq and proc surveymeans) to take into account the survey clusters, strata and weights (SAS version 9.3, SAS Institute, Cary, NC, USA). The weighting of sample data was used to produce estimates of the statistics they would have obtained if the entire sampling frame had been surveyed. Therefore, in order to make valid statistical inferences for the parameters of the population, statistical analyses in this study were taken into account the survey clusters, strata and weights.

A non-simple random sample may not represent the general population. As a complex multistage sample design, NHANES is a non-simple random sample. In addition, there are some other issues, such as non-response and non-coverage of the population in this study. If we estimate the population parameter with the non-weighted sample, we will get a biased estimation because the sample does not represent the target population. Therefore, to make sure that a sample is representative of the target population, we should calculate weights for all sample units. Then, we can get an unbiased estimate for this population after incorporating the weights in estimating the population parameter with sample [138, 158].

There were mainly two sample weights in NHANES for use; one was sample weights for in-home interview and another was sample weights for MEC exam. According to the rule “least common denominator” approach, we needed to select MEC exam sample weights because MEC exam sample weights had the least common denominator. Second, we needed to construct the combined sample weights for combined multi-year samples. We constructed the combined sample weights because we combined the samples from 2001 to 2006 in this study. The combined sample weights= 1/3 * original sample weights (for 01-02, 03-04 and 05-06 survey cycles). Third, we needed to take into account of cluster and strata design. For variance estimation, we also needed to take into account of cluster and strata for design in this study for unbiased estimation of variance with SAS survey procedures. Fourth, we needed to take into account of subsample analysis in the study; for example, we only analyzed the data for obesity and overweight adult participants. For proper estimation of variance, we needed to produce a variable for obesity and overweight adult participants=1, others=0; then use DOMAIN statements in the SAS survey procedure [160].

3.7.2. Statistical analysis methods

For Aim 1, chi-square tests were used to examine the associations between metabolic syndrome and race/ethnicity, gender, age and BMI status. The mean and standard deviation (SD) were used for estimating serum concentration of lycopene. In addition, logistic regression models were performed to evaluate the association between the prevalence of metabolic syndrome and serum concentration of lycopene and to calculate the odds ratios (ORs) and 95% Confidence Intervals (CIs) after adjusting for race, gender, age group, alcohol consumption, smoking and physical activity.

For Aim 2, chi-square tests were used to examine the associations between metabolic syndrome and race/ethnicity, gender, age and BMI status. The mean and SD was used

for estimating the ratio of serum concentration of lycopene to serum concentration of triglyceride. In addition, logistic regression models were performed to evaluate the association between the prevalence of metabolic syndrome and the ratio of serum concentration of lycopene to serum concentration of triglyceride and to calculate the ORs and 95% CIs after adjusting for race, gender, age group, alcohol consumption, smoking and physical activity.

For Aim 3, chi-square tests were used to examine the associations between the ratio of serum concentration of lycopene to serum concentration of triglyceride and race/ethnicity, gender, age group, BMI status, alcohol consumption, smoking status and physical activity. Chi-square tests were also used to examine the associations between the ratio of serum concentration of lycopene to serum concentration of triglyceride and mortality and disease-specific mortality. In addition, a Cox model was performed to evaluate the hazard ratios (HRs) and 95% CI of mortality for the ratio of serum concentration of lycopene to serum concentration of triglyceride adjusting for race/ethnicity, gender, age group, BMI status, alcohol consumption, smoking status, physical activity and cancers. Backward procedure was performed for model selection.

For Aim 4, physical activity levels were categorized into no physical activity, moderate physical activity and heavy physical activity. Chi-square tests were used to examine the associations between physical activity and the prevalence of metabolic syndrome. To examine the effects of serum lycopene on the prevalence of metabolic syndrome, participants were divided into three groups by tertile ranking method according to the ratio of serum lycopene to serum triglyceride. Chi-square tests were used to examine the associations between serum lycopene levels (first tertile group, second tertile group and third tertile group) and the prevalence of metabolic syndrome. To investigate the additive effect of physical activity and lycopene level on the prevalence of metabolic syndrome, Chi-

square tests were used—the tests examined the associations between all combinations between physical activity (no physical activity, moderate physical activity and heavy physical activity) and serum lycopene levels (first, second, and third tertile groups) and the prevalence of metabolic syndrome for each of the BMI group. In addition, logistic regression models were performed to evaluate the associations between physical activity, serum lycopene levels and the prevalence of metabolic syndrome for each of the BMI group. ORs and 95% CIs were calculated after adjusting for race, gender, age group, alcohol consumption, and smoking status. The mean ratio of serum lycopene to serum triglyceride by physical activity was also estimated.

For Aim 5, to examine the effect of physical activity on mortality, chi-square tests were used to examine the association between physical activity (no physical activity, moderate physical activity and heavy physical activity) and mortality. To examine the effects of serum lycopene on mortality, participants were divided into three groups by tertile ranking method according to the ratio of serum lycopene to serum triglyceride. Chi-square tests were used to examine the association between serum lycopene levels and mortality. To investigate the additive effect of physical activity and lycopene level on mortality, Chi-square tests were used to examine the associations between physical activity, serum lycopene levels and mortality. In addition, a Cox model was performed to evaluate the hazard ratios (HRs) and 95% CIs after adjusting for race, gender, age group, alcohol consumption, smoking status. The mean ratio of serum lycopene to serum triglyceride by physical activity was also estimated.

Statistical analyses were adjusted for clusters and strata of the complex sample design of the NHANES 2001-2006, using sample weights for aim 1, 2 and 4. Statistical analyses were not adjusted for clusters and strata of the complex sample de-sign of the NHANES

2001-2006, using sample weight for aim 3 and 5. A two-sided P-value < 0.05 was considered to be statistically significant for all statistical analysis.

3.8. Assumptions of the Study

We assumed that all participants truthfully responded to the survey questions. We also assumed that serum concentration of lycopene and serum concentration of triglyceride did not change from the interview (2001-2006) to death or December 31, 2011. In addition, serum samples, such as serum concentration of lycopene and serum concentration of triglyceride, were only available at baseline; we cannot estimate the effect of changes of serum lycopene and serum triglyceride on the risk of mortality over time because of the cross-sectional design of NHANES 2001-2006.

3.9. Summary

The study belonged to the population-based cross-sectional study design because the data for this study came from the NHANES. The combination of NHANES 2001–2006 included 15,431 participants who were at least 20 years of age (7,341 men and 8,090 women). The main outcomes were the prevalence of metabolic syndrome and mortality. The main exposures were serum concentration of lycopene or the ratio of serum lycopene to serum triglyceride. Statistical analyses in this study were performed using SAS Survey Procedures (i.e., proc surveyfreq and proc surveymeans) to take into account of survey clusters, strata and weights.

CHAPTER 4 RESEARCH FINDINGS

4.1. The Influence of BMI on the Association between Serum Lycopene and the Prevalence of Metabolic Syndrome

4.1.1. Demographic characteristics, BMI status, and serum lycopene level of adults with metabolic syndrome and those without metabolic syndrome

Of the 13,196 participants, 3,601 (27.3%) had a diagnosis of metabolic syndrome. The prevalence of each metabolic syndrome condition was the following: abdominal obesity (50.3%; 95% CI: 48.7-51.9) hypertriglyceridemia (32.4%; 95% CI: 31.2-33.5), low HDL-cholesterol (32.4%; 95% CI: 31.2-33.5), hypertension (30.8%; 95% CI: 29.5-32.0), and high fasting glucose (18.3%; 95% CI: 16.9-19.6). Demographic characteristics, the BMI status, and the serum lycopene level of adults with metabolic syndrome and those without metabolic syndrome are presented in Table 1. Individuals with metabolic syndrome tended to be older than those without metabolic syndrome. For example, 46.8% of those with metabolic syndrome were from the oldest age group (≥60 years) compared to 27.3% for those without metabolic syndrome. There was a significant difference in racial/ethnic characteristics between these two groups. For example, the metabolic syndrome group had a higher proportion of Mexican Americans than the non-metabolic syndrome group (23.4% vs. 19.7%). The percent of metabolic syndrome was substantially increased with increased BMI. Less than 10% of individuals in the metabolic syndrome group were classified as having a normal weight while about 40% of the non-metabolic syndrome group were in that category. Conversely, close to 60% of the metabolic group were in the obese category while about 20% of the non-metabolic group were in that category. The

mean of serum concentration of lycopene was significantly lower in participants with metabolic syndrome than participants without metabolic syndrome (0.38 umo/L vs. 0.42 umo/L).

Table 1. Demographic characteristics, BMI status, and serum lycopene level of adults with metabolic syndrome and those without metabolic syndrome

Variable	**Metabolic Syndrome (N=3,601)**		**No Metabolic Syndrome (N=9,595)**		**P-value**
	N	**% or Mean (SD)**	**N**	**% or Mean (SD)**	
Age (years)					
20-39	698	19.4	4,170	43.5	<0.0001
40-59	1,219	33.9	2,799	29.2	
≥ 60	1,684	46.8	2,626	27.3	
Sex					
Male	1,720	47.8	4,615	48.1	0.0897
Female	1,881	52.2	4,980	51.9	
Race					
NH White	1,978	54.9	4,912	51.2	<0.0001
NH African American	550	15.3	2,065	21.5	
Mexican American	844	23.4	1,887	19.7	
Other	229	6.4	731	7.6	
BMI group					
Normal weight	276	7.7	3,853	40.2	<0.0001
Overweight	1,258	34.9	3,461	36.1	
Obese	2,067	57.4	2,281	23.7	
Serum lycopene (umol/L)	3,601	0.38 (0.20)	9,595	0.42 (0.21)	<0.0001

NH: Non-Hispanic; N: number; SD: standard deviation.

4.1.2. Interaction between Serum Lycopene Concentration and BMI Status

The mean of serum concentration of lycopene was significantly lower in participants with metabolic syndrome than participants without metabolic syndrome. The mean serum concentration of lycopene was 0.206 umol/L (95% CI: 0.203-0.209) for the first tertile group, 0.387 umol/L (95% CI: 0.385-0.389) for the second tertile group and 0.642 umol/L (95% CI: 0.635-0.648) for the third tertile group. The prevalence of metabolic syndrome was significantly higher in the first tertile group [31.5% (95% CI: 29.9-32.9)] compared with

the second tertile group [25.5% (95% CI: 23.9-27.2)] and the third tertile group [22.9% (95% CI: 21.4-24.3)]. Table 2 shows the results of a multivariate logistic analysis. After adjusting for race, gender, age, alcohol consumption, smoking, physical activity and BMI status, there was still a significant association between metabolic syndrome and the levels of serum lycopene. Most importantly, there was a significant interaction effect between serum lycopene and BMI status on metabolic syndrome ($p<0.0001$).

Table 2. A multivariate logistic model for the association between the prevalence of metabolic syndrome and serum levels of lycopene

Effect	DF	Wald Chi-Square	P-value
Race	3	165.19	<.0001
Gender	1	8.10	0.0044
Age group	2	252.45	<.0001
Alcohol consumption	2	30.95	<.0001
Smoking status	2	19.23	<.0001
Physical activity	2	57.22	<.0001
Serum lycopene	2	21.58	<.0001
BMI	2	1370.15	<.0001
Serum lycopene* BMI	4	31.90	<.0001

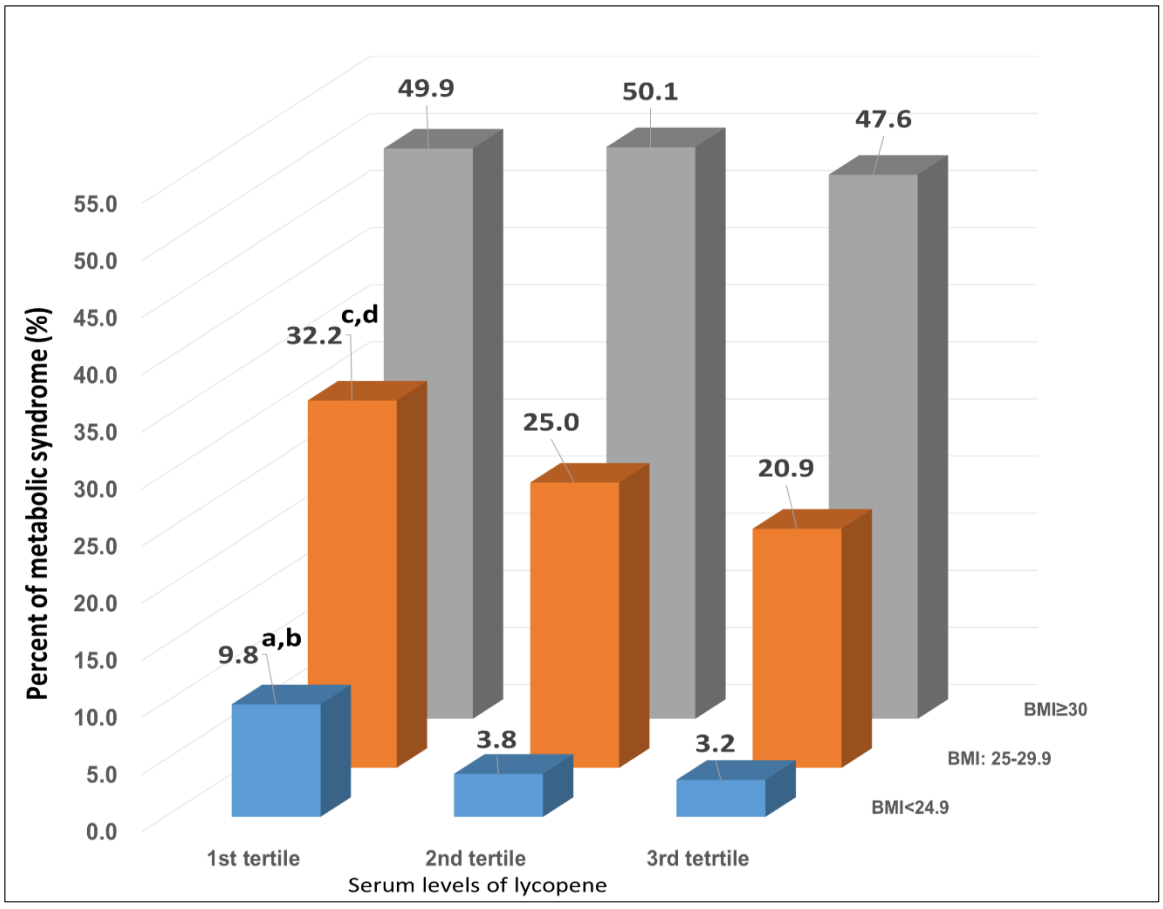
DF: degree of freedom; BMI: body mass index.

4.1.3. Stratification by BMI Status

BMI is an effect modifier of the association between serum lycopene and metabolic syndrome. With an effect modification, a stratified analysis is an appropriate method to examine the association between exposure and outcome. Figure 7 shows the associations between serum lycopene and metabolic syndrome stratified by BMI status. For normal weight participants, the prevalence of metabolic syndrome was significantly lower in the third tertile group [3.2%, 95% CI: 2.1-4.3] compared to the first tertile group [9.8%, 95% CI: 7.9-11.8], and also the prevalence of metabolic syndrome was significantly lower in the second tertile group [3.8%, 95% CI: 2.6-4.9] compared to the first tertile group [9.8%, 95% CI: 7.9-11.8]. For overweight participants, the prevalence of metabolic syndrome was significantly lower in the third tertile group [20.9%, 95% CI: 18.6-23.3] compared to the

first tertile group [32.2%, 95% CI: 29.3-35.0] and also the prevalence of metabolic syndrome was significantly lower in the second tertile group [25.0, 95% CI: 22.8-27.2] compared to the first tertile group [32.2%, 95% CI: 29.3-35.0]. However, for obese participants, there was no significant difference of the prevalence of metabolic syndrome among the first tertile group [49.9%, 95% CI: 47.5-52.3], the second tertile group [50.1%, 95% CI: 46.5-53.6] and the third tertile group [47.6%, 95% CI: 44.3-51.0].

Figure 7. The association between metabolic syndrome and serum concentration of lycopene stratified by BMI status



BMI: body mass index
[a] The prevalence of metabolic syndrome was significantly different ($p<0.05$) between the 1st and 2nd tertiles for BMI<24.9.
[b] The prevalence of metabolic syndrome was significantly different ($p<0.05$) between the 1st and 3rd tertiles for BMI<24.9.
[c] The prevalence of metabolic syndrome was significantly different ($p<0.05$) between the 1st and 2nd tertiles for BMI:25-29.9.
[d] The prevalence of metabolic syndrome was significantly different ($p<0.05$) between the 1st and 3rd tertiles for BMI:25-29.9.

After adjusting for race, gender, age, alcohol consumption, smoking and physical activity, the associations between metabolic syndrome and serum levels of lycopene remained significant for participants who were normal weight or overweight (Table 3).

Table 3. Multivariate logistic models for the associations between the prevalence of metabolic syndrome and serum levels of lycopene by BMI status

BMI	Serum Lycopene				
	1st Tertile	2nd Tertile		3rd Tertile	
	OR	OR*	95% CI	OR*	95% CI
<24.9	1	**0.56**	(0.34-0.90)	**0.51**	(0.31-0.85)
25-29.9	1	0.92	(0.76-1.11)	**0.76**	(0.59-0.97)
≥30	1	1.07	(0.90-1.28)	1.03	(0.87-1.22)

BMI: body mass index; OR: odds ratio; CI: confidence interval; *: Adjusted for race, gender, age, alcohol consumption, smoking and physical activity

4.1.4. Stratification by serum levels of lycopene and BMI status

We compared the mean serum concentration of lycopene and serum concentration of triglyceride by serum lycopene and BMI status. Table 4 shows the mean of serum concentration of lycopene and serum concentration of triglyceride stratified by serum levels of lycopene and BMI status. For normal weight participants, the mean serum concentration of lycopene was significantly higher in the third tertile group (0.63 umol/L) when compared to the first tertile group (0.20 umol/L). However, there was no significant difference of serum concentration of triglyceride between the third tertile group (1.20mmol/L) and the first tertile group (1.33mmol/L). For overweight participants, the mean serum concentration of lycopene was significantly higher in the third tertile group (0.65 umol/L) when compared to the first tertile group (0.21 umol/L). There was no significant difference of serum concentration of triglyceride between the third tertile group (1.88mmol/L) and the first tertile group (1.77mmol/L). For obese participants, the mean serum concentration of lycopene was significantly higher in the third tertile group (0.64 umol/L) when compared to the first tertile group (0.21 umol/L). Also, the serum concentration of triglyceride was significantly different between the third tertile group (2.08mmol/L) and the first tertile group (1.83mmol/L).

Table 4. The mean of serum concentration of lycopene and serum concentration of triglyceride stratified by serum levels of lycopene and BMI status

Tertile	Label	BMI								
		<24.9			25.0-29.9			≥30		
		N	Mean	95% CI	N	Mean	95% CI	N	Mean	95% CI
1st	Lycopene (umol/L)	1,307	0.20	(0.19-0.20)	1,547	0.21	(0.20-0.21)	1,534	0.21	(0.21-0.22)
	Triglycerides (mmol/L)	1,298	1.33	(1.17-1.48)	1,540	1.77	(1.67-1.87)	1,528	1.83	(1.74-1.92)
2nd	Lycopene (umol/L)	1,411	0.39	(0.38-0.39)	1,546	0.39	(0.38-0.39)	1,453	0.39	(0.39-0.39)
	Triglycerides (mmol/L)	1,407	1.14	(1.10-1.18)	1,542	1.63	(1.56-1.70)	1,451	2.04	(1.91-2.17)
3rd	Lycopene (umol/L)	1,411	0.63	(0.62-0.64)	1,626	0.65	(0.64-0.66)	1,361	0.64	(0.63-0.65)
	Triglycerides (mmol/L)	1,408	1.20	(1.16-1.25)	1,621	1.88	(1.74-2.01)	1,359	2.08	(1.98-2.19)

N: number; BMI: body mass index; CI: confidence interval.

4.2. An Association between the Lycopene to Triglyceride Ratio and the Prevalence of Metabolic Syndrome

4.2.1. Demographic characteristics, BMI status, and the ratio of serum lycopene to serum triglyceride of adults with metabolic syndrome and those without metabolic syndrome

Of the 13,154 participants, 3,596 (27.3%) had a diagnosis of metabolic syndrome. Table 5 shows the demographic characteristics, BMI status, and serum lycopene level of adults with metabolic syndrome and those without metabolic syndrome. Individuals with metabolic syndrome tended to be older than those without metabolic syndrome. For example, 46.7% of those with metabolic syndrome were in the oldest age group (≥60 years) compared to 27.3% for those without metabolic syndrome. There was a significant difference in racial/ethnic characteristics between these two groups. For example, the metabolic syndrome group had a higher proportion of Mexican Americans than the non-metabolic syndrome group (23.4% vs. 19.7%). The percent of metabolic syndrome was substantially higher among overweight and obese individuals compared to those with a normal weight. Less than 10% of the individuals with metabolic syndrome were classified as having a normal weight while about 40% of the non-metabolic syndrome group were in that category. Conversely, close to 60% of the metabolic group were in the obese category while about 20% of the non-metabolic groups were in that category. The mean ratio of serum concentration of lycopene to serum concentration of triglyceride was significantly lower in participants with metabolic syndrome than participants without metabolic syndrome.

Table 5. Demographic characteristics, BMI status, and the ratio of serum lycopene to serum triglyceride of adults with metabolic syndrome and those without metabolic syndrome

Variable	Metabolic Syndrome (N=3,596)		No Metabolic Syndrome (N=9,558)		P-value
	N	% or Mean (SD)	N	% or Mean (SD)	
Age (years)					
20-39	697	19.4	4,158	43.5	<0.0001
40-59	1,219	33.9	2,789	29.2	
≥60	1,680	46.7	2,611	27.3	
Sex					
Male	1,718	47.8	4,599	48.1	0.0925
Female	1,878	52.2	4,959	51.9	
Race					
NH White	1,975	54.9	4,893	51.2	<0.0001
NH African American	549	15.3	2,054	21.5	
Mexican American	843	23.4	1,883	19.7	
Other	229	6.4	728	7.6	
BMI group					
Normal weight	276	7.7	3,837	40.1	<0.0001
Overweight	1,254	34.9	3,449	36.1	
Obese	2,066	57.5	2,272	23.8	
Ratio of serum lycopene to serum triglyceride	3,596	0.20 (0.16)	9,558	0.42 (0.31)	<0.0001

NH: Non-Hispanic; N: number; SD: standard deviation.

4.2.2. An association between the ratio of serum lycopene to serum triglyceride and the prevalence of metabolic syndrome

The mean ratio of serum lycopene to serum triglyceride was significantly lower in individuals with metabolic syndrome than individuals without metabolic syndrome. To further estimate the association between the prevalence of metabolic syndrome and the ratio of serum lycopene to serum triglyceride, three groups of participants were divided by tertile rank method according to the ratio of serum lycopene to serum triglyceride. The mean ratio of serum lycopene to serum triglyceride was 0.12 (95% CI: 0.12-0.12) for the first tertile group, 0.28 (95% CI: 0.28-0.29) for the second tertile group and 0.67 (95% CI: 0.66-0.68) for the third tertile group.

The prevalence of metabolic syndrome was significantly higher in the first tertile group [52.7% (95% CI: 50.9-54.5)] compared to the second tertile group [24.7% (95% CI: 23.1-26.4)] and the third tertile group [7.0% (95% CI: 5.9-8.1)]. To adjust for possible confounding effects, a multivariate logistic analysis was performed to evaluate the associations between the prevalence of metabolic syndrome and the ratio of serum lycopene to serum triglyceride (Table 6). After adjusting for race, gender, age, alcohol consumption, smoking, physical activity and BMI status, there was still a significant association between metabolic syndrome and the ratio of serum lycopene to serum triglyceride.

Table 6. A multivariate logistic model for the associations between the prevalence of metabolic syndrome and the ratio of serum lycopene to serum triglyceride

Effect	DF	Wald Chi-Square	P-value
Race	3	56.64	<.0001
Gender	1	1.55	0.2135
Age group	2	109.83	<.0001
Alcohol consumption	2	15.61	0.0004
Smoking status	2	2.82	0.2437
Physical activity	2	16.27	0.0003
Ratio of Serum lycopene to serum triglyceride	2	425.04	<.0001
BMI	2	546.44	<.0001
Ratio of Serum lycopene to serum triglyceride *BMI	4	16.216	0.0027

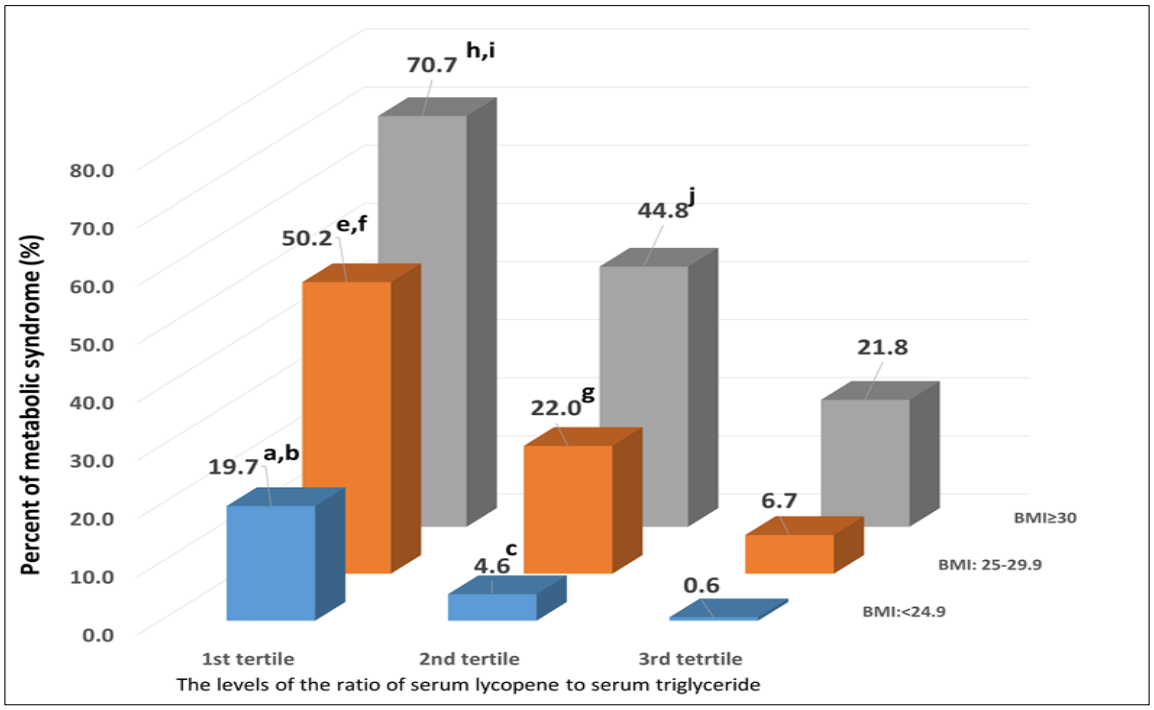
DF: degree of freedom; BMI: body mass index.

4.2.3. Stratification by BMI status

To study the effect of the ratio of serum lycopene to serum triglyceride on the prevalence of metabolic syndrome among obese participants, associations between the ratio of serum lycopene to serum triglyceride and metabolic syndrome were examined with a stratified analysis by BMI status (Figure 8). For each BMI status, there was a significant difference in the prevalence of metabolic syndrome based on the ratio of serum lycopene to serum triglyceride. For example, within the normal weight group, the prevalence of metabolic syndrome was the significantly higher among individuals in the first tertile group [19.7%, 95% CI: 16.2-23.2] compared to the second tertile group [4.6%, 95% CI: 3.3-5.8].

Similarly, the prevalence of metabolic syndrome was significantly higher among the second tertile group compared to the third tertile group [0.6%, 95% CI: 0.2-1.0]. The same types of significant trends were observed for other BMI groups as well.

Figure 8. The association between metabolic syndrome and the ratio of serum lycopene to serum triglyceride stratified by BMI status



BMI: body mass index;
[a] The prevalence of metabolic syndrome was significantly different (p<0.05) between the 1st and 2nd tertiles for BMI<24.9.
[b] The prevalence of metabolic syndrome was significant different (p<0.05) between the 1st tertile and 3rd tertile for BMI<24.9.
[c] The prevalence of metabolic syndrome was significant difference (p<0.05) between the 2nd and 3rd tertile for BMI<24.9.
[e] The prevalence of metabolic syndrome was significantly different (p<0.05) between the 1st and 2nd tertiles for BMI:25-29.9.
[f] The prevalence of metabolic syndrome was significant different (p<0.05) between the 1st tertile and 3rd tertile for BMI: 25-29.9.
[g] The prevalence of metabolic syndrome was significant difference (p<0.05) between the 2nd and 3rd tertile for BMI:25-29.9.
[h] The prevalence of metabolic syndrome was significantly different (p<0.05) between the 1st and 2nd tertiles for BMI≥30.
[i] The prevalence of metabolic syndrome was significant different (p<0.05) between the 1st tertile and 3rd tertile for BMI≥30.
[j] The prevalence of metabolic syndrome was significant difference (p<0.05) between the 2nd and 3rd tertile for BMI≥30.

Multivariate logistic analyses were performed to evaluate the associations between the prevalence of metabolic syndrome and the ratio of serum lycopene to serum triglyceride for each BMI status group (Table 7). After adjusting for race, gender, age, alcohol consumption, smoking and physical activity, there were still significant associations between metabolic syndrome and the ratio of serum lycopene to serum triglyceride for participants who were normal weight, overweight or obese. In addition, the magnitude of odds ratio of metabolic syndrome was lower in the 3rd tertile participants who were normal weight [0.035, 95% CI: 0.016-0.078] than that in participants who were obese [0.131, 95% CI: 0.104-0.167].

Table 7. Logistic models for the associations between the prevalence of metabolic syndrome and the ratio of serum lycopene to serum triglyceride by BMI status

BMI	Ratio of Serum Lycopene to Serum Triglyceride				
	1st Tertile	2nd Tertile		3rd Tertile	
	OR	OR*	95% CI	OR*	95% CI
<24.9	1	0.243	(0.148-0.399)	0.035	(0.016-0.078)
25-29.9	1	0.289	(0.238-0.351)	0.083	(0.062-0.112)
≥30	1	0.359	(0.301-0.428)	0.131	(0.104-0.167)

BMI: body mass index; OR: odds ratio; CI: confidence interval; *: Adjusted for race, gender, age, alcohol consumption, smoking and physical activity

4.2.4. Stratification by serum levels of lycopene and BMI status

With the ratio of serum concentration of lycopene to serum concentration of triglyceride, the mean of serum concentration of lycopene was significantly increased from the first tertile groups to the third tertile groups for all BMI status (Table 8). Very importantly, the mean of serum concentration of triglyceride was significantly decreased from the first tertile groups to the third tertile groups for all BMI status. Therefore, we can get enough high relative serum concentration of lycopene for obese participants.

Table 8. The mean of serum concentration of lycopene and serum concentration of triglyceride stratified by serum levels of lycopene and BMI status

Rank	Label	BMI								
		<24.9			25.0-29.9			≥30		
		N	Mean	95% CI	N	Mean	95% CI	N	Mean	95% CI
1st	lycopene (umol/L)	917	0.23	(0.21-0.24)	1,673	0.30	(0.29-0.31)	1,794	0.30	(0.29-0.32)
	Triglycerides (mmol/L)	917	2.19	(1.93-2.45)	1,673	2.89	(2.73-3.04)	1,794	2.92	(2.81-3.03)
2nd	lycopene (umol/L)	1,283	0.38	(0.37-0.39)	1,580	0.44	(0.43-0.45)	1,522	0.44	(0.43-0.45)
	Triglycerides (mmol/L)	1,283	1.32	(1.29-1.36)	1,580	1.60	(1.55-1.65)	1,522	1.66	(1.61-1.70)
3rd	lycopene (umol/L)	1,913	0.53	(0.52-0.54)	1,450	0.57	(0.56-0.58)	1,022	0.57	(0.56-0.59)
	Triglycerides (mmol/L)	1,913	0.82	(0.80-0.84)	1,450	0.95	(0.92-0.98)	1,022	1.00	(0.97-1.03)

BMI: body mass index; N: number; CI: confidence interval.

4.3. Increased Levels of Serum Lycopene is Associated with Decreased Mortality in People with Metabolic Syndrome

4.3.1. Demographic characteristics of participants with metabolic syndrome by lycopene status

To examine the effect of serum lycopene on survival, three groups of participants with metabolic syndrome were divided by tertile rank method based on the ratio of serum lycopene to serum triglyceride. The mean ratio of serum concentration of lycopene to serum concentration of triglyceride was 0.078 (95% CI: 0.076-0.080) in the first tertile group (832 participants), 0.167 (95% CI: 0.165-0.169) in the second tertile group (832 participants) and 0.365 (95% CI: 0.349-0.381) in the third tertile group (832 participants). The demographic characteristics among the three tertile groups were listed in Table 9.

The distribution of race/ethnicity was significantly different among the three tertile groups. The percent of African American (21.0%) was higher in the third tertile group than that (9.3%) in the first tertile group while the percent of Mexican American (17.7%) was lower in the third tertile group than that (30.9%) in the first tertile group. The percent of females (52.9%) was higher in the third tertile group than that (48.0%) in the first tertile group. There was a lower percent of participants aged 60 years and older (30.3%) in the third tertile group than that (42.4%) in the first tertile group. There were no significant differences among the groups in relation to alcohol consumption. The percent of current smokers (25.6%) was higher in the first tertile group than that (19.3%) in the third tertile group. There were a higher percentage of participants with heavy physical activity in the third tertile group (27.8%) than that (18.8%) in the first tertile group.

Table 9. Demographic characteristics of participants with metabolic syndrome by serum levels of lycopene

Variable	1st Tertile (N=832)		2nd Tertile (N=832)		3rd Tertile (N=832)		P-value
	N	Percent	N	Percent	N	Percent	
Race							
NH White	433	52.0	497	59.7	464	55.8	<0.0001
NH African American	77	9.3	85	10.2	175	21.0	
Mexican American	257	30.9	193	23.2	147	17.7	
Other	65	7.8	57	6.9	46	5.5	
Gender							
Male	433	52.0	383	46.0	392	47.1	0.0328
Female	399	48.0	449	54.0	440	52.9	
Age (years)							
20-39	181	21.8	217	26.1	249	29.9	<0.0001
40-59	298	35.8	309	37.1	331	39.8	
≥60	353	42.4	306	36.8	252	30.3	
BMI group							
Normal weight	64	7.9	38	4.7	49	6.0	0.0006
Overweight	309	38.1	298	36.5	254	30.8	
Obese	439	54.1	481	58.9	521	63.2	
Alcohol consumption							
No	261	33.0	276	34.9	257	32.7	0.5077
Moderate	304	38.4	287	36.3	280	35.6	
Heavy	226	28.6	228	28.8	250	31.8	
Smoking							
No	383	46.3	398	48.0	450	54.2	0.0067
Past	233	28.1	241	29.0	221	26.6	
Current	212	25.6	191	23.0	160	19.3	
Physical activity							
No	416	50.0	370	44.5	317	38.1	<0.0001
Moderate	259	31.2	267	32.1	284	34.1	
Heavy	156	18.8	195	23.4	231	27.8	

NH: Non-Hispanic; N: number; BMI: body mass index.

4.3.2. Mortality by lycopene status in adults with metabolic syndrome

The NHANES 2001-2006 Linked Mortality File provided mortality information for participants involved in NHANES interview (2001-2006) through December 31, 2011. Of the 832 participants with metabolic syndrome in the first tertile group, 83 participants had died. Of the 832 participants with metabolic syndrome in the second tertile group, 50 participants had died. Of the 832 participants with metabolic syndrome in the third tertile group, 43 participants died. The proportion of individuals died was significantly lower in the third

tertile group (5.2%, 95% CI: 3.7-6.7) or in the second tertile group (6.0%, 95% CI: 4.4-7.6) compared to the first tertile group (10.0%, 95% CI: 7.9-12.0) among participants with metabolic syndrome.

4.3.3. An association between the serum lycopene and mortality in adults with metabolic syndrome

We conducted a Cox model to estimate the hazard ratios (HR) and 95% CI of mortality adjusting for race, gender, age groups, BMI status, smoking status, alcohol consumption, physical activity and cancers. The final model included race, age group, BMI status, smoking status, physical activity and serum levels of lycopene. Compared with the first tertile group (HR=1.0), both the third tertile (HR=0.63, 95% CI: 0.43-0.91) and the second tertile groups (HR=0.63, 95% CI: 0.43-0.94) had a significantly lower hazard ratios of mortality for participants with metabolic syndrome after adjusting for these variables (Table 10).

Table 10. Cox models for the associations between mortality and serum levels of lycopene for participants with metabolic syndrome

Serum Lycopene	Hazard Ratio*	95% CI
1st tertile	1.0	N/A
2nd tertile	0.63	(0.43-0.94)
3rd tertile	0.63	(0.43-0.91)

CI: confidence interval; N/A: not applicable; *Adjusted for race, gender, age groups, BMI status, smoking status, alcohol consumption, physical activity and cancers.

4.4. An Additive Effect of Physical Activity and Serum Lycopene on the Prevalence of Metabolic Syndrome among Overweight and Obese Adults

4.4.1. Independent effect of physical activity

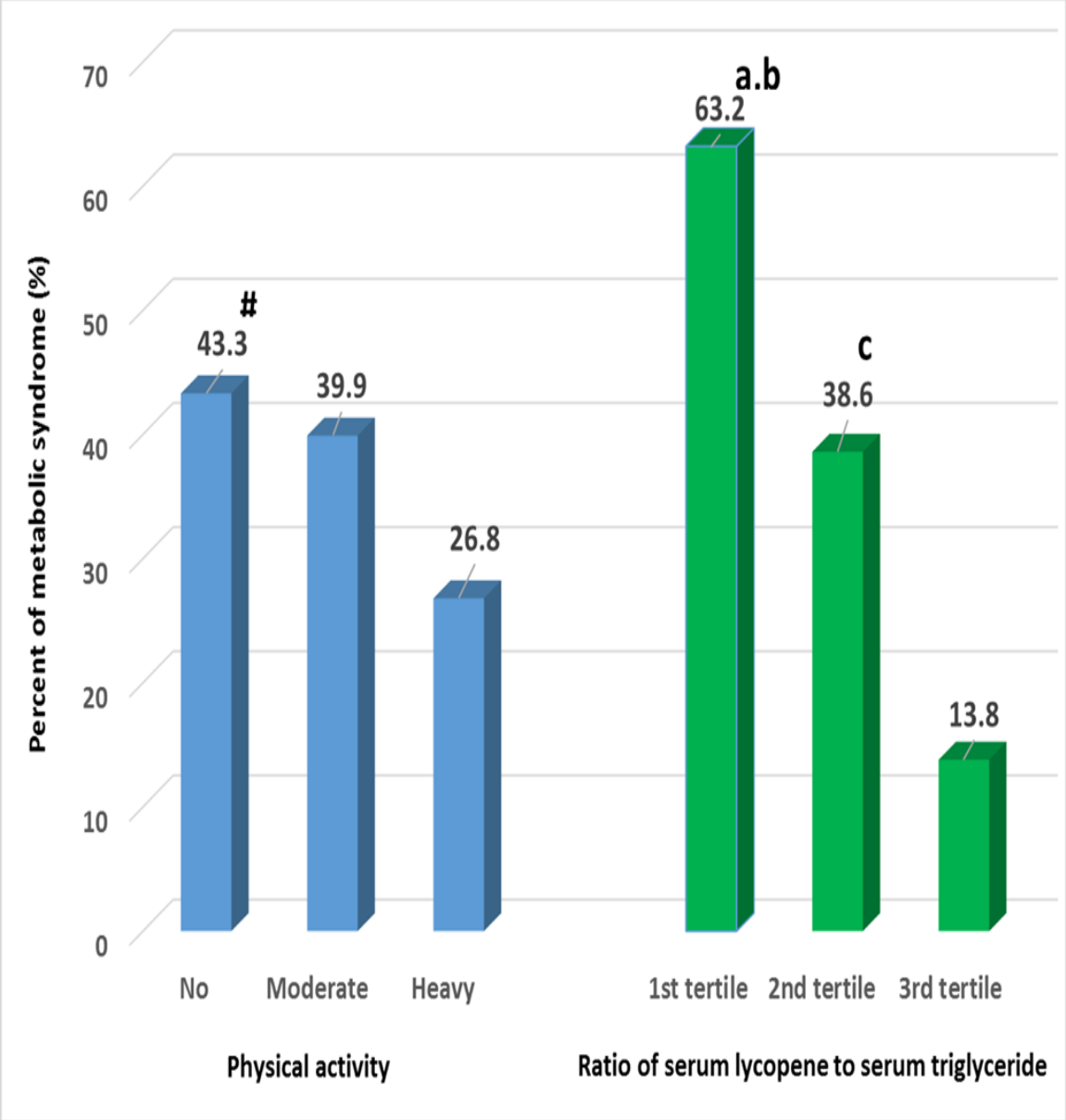
Of the 9,038 participants who were overweight or obese, there were 3,319 (36.7%) participants with metabolic syndrome. The prevalence of metabolic syndrome varied according to the levels of physical activity (Figure 9). The prevalence of metabolic syndrome

in participants with heavy physical activity (26.8%, 95% CI 24.9-28.7) was significantly lower compared to participants with no physical activity (43.3%, 95% CI 41.4-45.2).

4.4.2. Independent effect of serum lycopene

To estimate the association between the prevalence of metabolic syndrome and the ratio of serum lycopene to serum triglyceride, participants were divided by tertile rank method according to the ratio of serum lycopene to serum triglyceride. The mean ratio of serum lycopene to serum triglyceride was 0.107 (95% CI: 0.105-0.109) for the first tertile group, 0.249 (95% CI: 0.246-0.252) for the second tertile group and 0.592 (95% CI: 0.580-0.605) for the third tertile group. There was a significantly dose-dependent decrease the percent of metabolic syndrome from the first tertile group (63.2%, 95% CI: 61.2-65.2), the second tertile group (38.6%, 95% CI: 36.6-40.5) to the third tertile group (13.8%, 95% CI: 12.3-15.3) (Figure 9).

Figure 9. The association between metabolic syndrome and physical activity or the ratio of serum lycopene to serum triglyceride



[#] The prevalence of metabolic syndrome was significantly different ($p<0.05$) between the no physical activity and heavy physical activity.
[a] The prevalence of metabolic syndrome was significantly different ($p<0.05$) between the 1st and 2nd tertiles.
[b] The prevalence of metabolic syndrome was significant different ($p<0.05$) between the 1st tertile and 3rd tertile.
[c] The prevalence of metabolic syndrome was significant difference ($p<0.05$) between the 2nd and 3rd tertile.

4.4.3. An additive effect of physical activity and serum lycopene

To estimate the additive effect of physical activity and serum lycopene on the prevalence of metabolic syndrome, we combined all the levels of physical activity and the levels

of serum lycopene. There was a significant interaction effect between the combined physical activity and serum lycopene and BMI status on the prevalence of metabolic syndrome. BMI is an effect modifier of the association between the combined physical activity and serum lycopene and metabolic syndrome. Therefore, we showed the additive effects of physical activity and serum lycopene stratified by BMI status, overweight and obesity (Table 11).

For overweight participants, the prevalence of metabolic syndrome was significantly reduced with increased levels of serum lycopene for all physical activity levels. For example, the prevalence of metabolic syndrome reduced from 54.3% in the first tertile to 11.6% in the third tertile for participants without physical activity. The prevalence of metabolic syndrome reduced from 53.5% in the first tertile to 8.6% in the third tertile for participants with moderate physical activity. The prevalence of metabolic syndrome reduced from 39.0% in the first tertile to 2.6% in the third tertile for participants with heavy physical activity.

The prevalence of metabolic syndrome was significantly reduced in participants with heavy physical activity compared to participants without physical activity for all levels of serum lycopene. For example, in the first tertile, the prevalence of metabolic syndrome reduced from 54.3% among participants without physical activity to 39.0% among participants with heavy physical activity. In the second tertile, the prevalence of metabolic syndrome reduced from 23.9% among participants without physical activity to 15.1% among participants with heavy physical activity. In the third tertile, the prevalence of metabolic syndrome reduced from 11.6% among participants without physical activity to 2.6% among participants with heavy physical activity. Therefore, there was an additive effect of physical activity and serum lycopene on metabolic syndrome.

For obese participants, the prevalence of metabolic syndrome was still significantly reduced with increased levels of serum lycopene for all physical activity status. For example, the prevalence of metabolic syndrome reduced from 69.6% in the first tertile to 19.8% in the third tertile for participants without physical activity. The prevalence of metabolic syndrome reduced from 73.8% in the first tertile to 24.0% in the third tertile for participants with moderate physical activity. The prevalence of metabolic syndrome reduced from 68.3% in the first tertile to 21.5% in the third tertile for participants with heavy physical activity.

However, the prevalence of metabolic syndrome was not significantly different between participants with heavy physical activity and participants without physical activity for all levels of serum lycopene. For example, in the first tertile, the prevalence of metabolic syndrome was not different between participants without physical activity (69.6%) and participants with heavy physical activity (68.3%). In the second tertile, the prevalence of metabolic syndrome was not different between participants without physical activity (46.2%) and participants with heavy physical activity (42.2%). In the third tertile, the prevalence of metabolic syndrome was not different between participants without physical activity (19.8%) and participants with heavy physical activity (21.5%).Therefore, there was no an additive effect of physical activity and serum lycopene on metabolic syndrome.

Table 11. The prevalence of metabolic syndrome by the combination of the levels of physical activity and the levels of serum lycopene stratified by BMI status

BMI Status	**Physical Activity**	**Serum Lycopene**					
		1st Tertile		**2nd Tertile**		**3rd Tertile**	
		%	**95% CI**	**%**	**95% CI**	**%**	**95% CI**
Overweight	None	54.3	(49.0-59.7)	23.9	(19.7-28.2)	11.6	(7.5-15.7)
	Moderate	53.5	(48.1-59.0)	27.9	(23.8-32.1)	8.6	(5.1-12.1)
	Heavy	39.0	(33.1-45.0)	15.1	(10.8-19.4)	2.6	(1.3-3.9)
Obesity	None	69.6	(65.9-73.2)	46.2	(41.6-50.9)	19.8	(13.7-25.9)
	Moderate	73.8	(69.5-78.1)	45.2	(39.5-51.0)	24.0	(18.6-29.5)
	Heavy	68.3	(62.9-73.6)	42.2	(36.2-48.3)	21.5	(15.1-27.9)

BMI: body mass index; CI: confidence interval.

4.4.4. Logistic regression results

Logistic regression analyses were performed to evaluate the association between the prevalence of metabolic syndrome and the combination of the levels of physical activity and the levels of serum lycopene, and to calculate the ORs and 95% CI. Table 12 show the ORs and 95%CI after adjusting for race, gender, age group, alcohol consumption and smoking status in the risk of metabolic syndrome (Table 12).

For overweight participants, after adjusting for race, gender, age group, alcohol consumption and smoking status, the magnitude of OR was significantly reduced with increased levels of serum lycopene for all physical activity levels. For example, the magnitude of OR reduced from 1 in the first tertile to 0.12 (95% CI: 0.07-0.19) in the third tertile for participants without physical activity. The magnitude of OR reduced from 0.94 (95% CI: 0.67-1.32) in the first tertile to 0.08 (95% CI: 0.05-0.13) in the third tertile for participants with moderate physical activity. The magnitude of OR reduced from 0.65 (95% CI: 0.47-0.90) in the first tertile to 0.03 (95% CI: 0.02-0.06) in the third tertile for participants with heavy physical activity. The magnitude of OR was also significantly reduced with increased level of physical activity for serum lycopene levels. For example, in the first tertile, the magnitude of OR reduced from 1 among participants without physical activity to 0.65 (95% CI: 0.47-0.90) among participants with heavy physical activity. In the third tertile, the magnitude of OR reduced from 0.12 (95% CI: 0.07-0.19) among participants without physical activity to 0.03 (95% CI: 0.02-0.06) among participants with heavy physical activity. Therefore, therefore, there was an additive effect of physical activity and serum lycopene on metabolic syndrome.

For obese participants, after adjusting for race, gender, age group, alcohol consumption and smoking status, the magnitude of OR was significantly reduced with increased levels of serum lycopene for all physical activity levels. For example, the magnitude of OR

reduced from 1 in the first tertile to 0.12 (95% CI: 0.08-0.17) in the third tertile for participants without physical activity. The magnitude of OR reduced from 1.16 (95% CI: 0.87-1.56) in the first tertile to 0.15 (95% CI: 0.11-0.22) in the third tertile for participants with moderate physical activity. The magnitude of OR reduced from 0.92 (95% CI: 0.67-1.27) in the first tertile to 0.14 (95% CI: 0.09-0.20) in the third tertile for participants with heavy physical activity. However, the magnitude of OR was not significantly different between participants with heavy physical activity and participants without physical activity for all levels of serum lycopene. For example, in the first tertile, the magnitude of OR was not different between participants without physical activity (1) and participants with heavy physical activity (0.92, 95% CI: 0.67-1.27). In the second tertile, the magnitude of OR was not different between participants without physical activity (0.37, 95% CI: 0.28-0.50) and participants with heavy physical activity (0.35, 95% CI: 0.26-0.47). In the third tertile, the magnitude of OR was not different between participants without physical activity (0.12, 95% CI: 0.08-0.17) and participants with heavy physical activity (0.14, 95% CI: 0.09-0.20). Therefore, there was no an additive effect of physical activity and serum lycopene on metabolic syndrome.

Table 12. Multivariate logistic models for the associations between the prevalence of metabolic syndrome by the combination of the levels of physical activity and the levels of serum lycopene stratified by BMI status

BMI Status	Physical Activity	Serum Lycopene					
		1st Tertile		2nd Tertile		3rd Tertile	
		OR*	95% CI	OR*	95% CI	OR*	95% CI
Overweight	None	1	N/A	0.28	(0.19-0.39)	0.12	(0.07-0.19)
	Moderate	0.94	(0.67-1.32)	0.29	(0.21-0.42)	0.08	(0.05-0.13)
	Heavy	0.65	(0.47-0.90)	0.18	(0.12-0.26)	0.03	(0.02-0.06)
Obesity	None	1	N/A	0.37	(0.28-0.50)	0.12	(0.08-0.17)
	Moderate	1.16	(0.87-1.54)	0.38	(0.29-0.51)	0.15	(0.11-0.22)
	Heavy	0.92	(0.67-1.27)	0.35	(0.26-0.47)	0.14	(0.09-0.20)

BMI: body mass index; OR: odds ratio; CI: confidence interval; N/A: not applicable; * Adjusted for race, gender, age group, alcohol consumption and smoking status in the risk of metabolic syndrome.

4.4.5. The mean ratio of serum lycopene to serum triglyceride by physical activity

The mean ratio of serum lycopene to serum triglyceride was significantly higher in participants with heavy physical activity than participants without physical activity for all BMI status (Table 13). For example, among overweight participants, the ratio increased from 0.31 (95% CI: 0.30-0.33) in participants with no physical activity to 0.42 (95% CI: 0.40-0.44) in participants with heavy physical activity. For example, among obese participants, the ratio increased from 0.27 (95% CI: 0.25-0.28) in participants with no physical activity to 0.34 (95% CI: 0.33-0.36) in participants with heavy physical activity. The mean ratio of serum lycopene to serum triglyceride was significantly higher with increased levels of physical activity.

Table 13. The mean ratio of serum lycopene to serum triglyceride by physical activity

BMI Status	Physical Activity	N	Mean Ratio of Serum Lycopene to Serum Triglyceride	95% CI
Overweight	None	1,883	0.31	(0.30-0.33)
	Moderate	1,414	0.35	(0.33-0.37)
	Heavy	1,403	0.42	(0.40-0.44)
Obesity	None	1,936	0.27	(0.25-0.28)
	Moderate	1,366	0.29	(0.28-0.31)
	Heavy	1,036	0.34	(0.33-0.36)

BMI: body mass index; N: number; CI: confidence interval.

4.5. An Additive effect of Physical Activity and Serum Lycopene on Mortality in Adults with Metabolic Syndrome

4.5.1. The mortality among adults with metabolic syndrome by physical activity

The combination of NHANES 2001–2006 included 3,868 participants who were at least 20 years of age with metabolic syndrome. Participants who had missing information on serum lycopene and serum triglyceride and physical activity (n=176) were excluded. In addition, individuals who had diabetes or heart disease or stroke (n=1,130) and/or who

died from diabetes or heart disease or stroke (n=67) were excluded. As a result, the final sample included 2,495 individuals.

The percent of individuals who had died was significantly reduced in participants with heavy physical activity (3.1%) compared to the participants with no physical activity (9.3%) (Table 14).

Table 14. The percentage of individuals with metabolic syndrome who had died: Stratification by physical activity level

Physical Activity	N	% of Individuals Died	95% CI
None	1,103	9.3	(7.6-11.1)
Moderate	810	6.7	(4.9-8.3)
Heavy	582	3.1	(1.7-4.5)

N: number; CI: confidence interval.

4.5.2. Mortality by the levels of serum lycopene among adults with metabolic syndrome

To examine the effect of serum lycopene on death, participants with metabolic syndrome were divided by tertile rank method based on the ratio of serum lycopene to serum triglyceride. The mean ratio of serum lycopene to serum triglyceride was 0.078 (95% CI: 0.076-0.080) in the first tertile group, 0.167 (95% CI: 0.165-0.169) in the second tertile group and 0.365 (95% CI: 0.349-0.381) in the third tertile group. The proportion of individuals who had died was significantly reduced in the third tertile group (5.2%) compared to the first tertile group (9.9%) among participants with metabolic syndrome (Table 15).

Table 15. The percentage of individuals with metabolic syndrome who had died: Stratified by serum lycopene levels

Serum Lycopene	N	% of Individuals Died	95% CI
1st tertile	831	9.9	(7.8-11.9)
2nd tertile	832	6.0	(4.4-7.6)
3rd tertile	832	5.2	(3.7-6.7)

N: number; CI: confidence interval.

4.5.3. Mortality by physical activity and serum lycopene among adults with metabolic syndrome

Due to the second tertile group and the third tertile group having similar mortality rate, we combined these two groups. The proportion of individuals who died was significantly reduced in participants with higher levels of serum lycopene and heavy physical activity (2.8%, 95% CI: 1.2-4.4) compared to participants with higher levels of serum lycopene and no physical activity (7.7%, 95% CI: 5.7-9.7) (Table 16). The proportion of individuals who had died was similar between participants with higher levels of serum lycopene and heavy physical activity (2.8%, 95% CI: 1.2-4.4) and participants with lower levels of serum lycopene and heavy physical activity (3.8%, 95% CI: 0.8-6.8).

Table 16. The proportion of individuals who had died in metabolic syndrome by physical activity and serum lycopene levels

Physical Activity	**Serum Lycopene**					
	1st Tertile			**2nd and 3rd Tertile**		
	N	**% Died**	**95% CI**	**N**	**% Died**	**95% CI**
None	416	12.0	(8.9-15.1)	687	7.7	(5.7-9.7)
Moderate	259	10.0	(6.4-13.7)	551	5.1	(3.2-6.9)
Heavy	156	3.8	(0.8-6.8)	426	2.8	(1.2-4.4)

N: number; CI: confidence interval.

4.5.4. Additive effects of physical activity and serum lycopene on mortality among adults with metabolic syndrome

We conducted Cox analyses to estimate hazard ratios and 95% CIs of mortality after adjusting for race, gender, age, smoking status, alcohol consumption (Table 17). There was a significant effect of serum lycopene on mortality for participants with metabolic syndrome. However, there was no a significant additive effect of physical activity and serum lycopene on mortality.

Table 17. Hazard ratios (HRs) and 95% CI of mortality in metabolic syndrome by combing of physical activity and lycopene levels in the multivariate logistic regression models

Physical Activity	Serum Lycopene Level			
	1st Tertile		2nd & 3rd Tertiles	
	HR*	95% CI	HR*	95% CI
None	1	N/A	0.651	(0.430-0.985)
Moderate	0.663	(0.398-1.106)	0.395	(0.241-0.650)
Heavy	0.506	(0.215-1.194)	0.317	(0.162-0.621)

CI: confidence interval.

4.5.5. The mean ratio of serum lycopene to serum triglyceride by physical activity among adults with metabolic syndrome

The mean ratio of serum lycopene to serum triglyceride was significantly higher in participants with heavy physical activity than participants without physical activity (Table 18). For example, the ratio increased from 0.19 (95% CI: 0.18-0.20) in participants without physical activity to 0.23 (95% CI: 0.22-0.24) in participants with heavy physical activity.

Table 18. The mean ratio of serum lycopene to serum triglyceride by physical activity

Physical Activity	N	Mean of Ratio of Serum Lycopene to Serum Tri-glyceride	95% CI
None	1,103	0.19	(0.18-0.20)
Moderate	810	0.22	(0.20-0.23)
Heavy	582	0.23	(0.22-0.24)

N: number; CI: confidence interval.

4.6. Summary of Findings

In this chapter, we presented the main findings from the analyses of NHANES data. First, we found that BMI had an important influence on the association between serum lycopene and metabolic syndrome. A higher serum concentration of lycopene was significantly associated with a lower prevalence of metabolic syndrome. However, these associations were only significant for normal weight and overweight participants, but not for obese participants. Secondly, the association between the ratio of serum lycopene to

serum triglyceride and the prevalence of metabolic syndrome was significant not only for normal weight and overweight participants, but also for obese participants.

Thirdly, a high level of serum lycopene was significantly associated with a decreased mortality among participants with metabolic syndrome. Metabolic syndrome participants with a higher level of serum lycopene (the mean ratio of serum lycopene to serum triglyceride=0.364) had a significantly smaller proportion of death ($p<0.0001$) than those with a lower level of serum lycopene (the mean ratio=0.077). Even after adjusting for race, age, BMI status, smoking and physical activity, metabolic syndrome participants with a higher level of serum lycopene still had a significantly reduced hazard ratio of mortality than those with a lower level of serum lycopene ($p<0.05$). Fourthly, physical activity and serum lycopene had an additive effect on the prevalence of metabolic syndrome for overweight individuals but not for obese individuals. Fifthly, there was not an additive effect of physical activity and serum lycopene on mortality.

CHAPTER 5 DISCUSSION

5.1. Discussion

5.1.1. Health effects of lycopene on obese individuals

Metabolic syndrome is an important public health issue. About one in three United States adult aged 20 years and older has metabolic syndrome [2]. Metabolic syndrome is a direct risk factor for chronic diseases, such as cardiovascular diseases, diabetes mellitus, stroke and cancers [1, 3-5 and 160]. The growing prevalence of metabolic syndrome is strongly related to the increasing prevalence of overweight and obesity [2, 12]. Therefore, preventing overweight and obesity is an important strategy to reduce the prevalence of metabolic syndrome. However, interventions related to overweight and obesity have not achieved expected results; more than one-third of adults and 17% of youth in the United States are obese [75]. Therefore, reducing the development of metabolic syndrome in individuals who are overweight and obese may be an innovative approach to reduce the total burden of chronic diseases in the general population.

As a natural nutrient, the higher level of lycopene is significantly associated with the lower prevalence of metabolic syndrome in the general population [23-25]. The biological mechanisms by which lycopene reduces the risk of metabolic syndrome include alleviating oxidative stress and decreasing inflammation [18-22, 126]. Studies show that the increased risk of metabolic syndrome in individuals who are overweight and obese contributes to increased inflammation and oxidative stress production [11, 13 and 161]. Therefore, increasing serum concentration of lycopene is a potential strategy to decrease the risk of developing metabolic syndrome in individuals who are overweight and obese. However, despite the cumulating evidence to support the health effects of lycopene, it is

not clear whether lycopene has similar effects among individuals who are overweight or obese.

For this reason, recently, more studies about the health effects of lycopene began to focus on individuals who were overweight and obese [32-37]. However, the findings from these studies have been inconclusive. There are two potential reasons for conflicting results among the studies: one potential reason is that the participants had different BMI levels [34, 35], and the other reason is that the participants had different serum concentrations of lycopene [33, 37].

The same “high” serum concentrations of lycopene for individuals with a lower BMI level may not produce the same health effects in individuals with a higher BMI level because there is more inflammation and oxidative stress production in individuals who are obese or overweight [34, 35]. Our results strongly supported the idea that with the same serum concentration of lycopene, lycopene is significantly associated with the prevalence of metabolic syndrome only for normal weight and overweight individuals, but not for obese individuals. The biological mechanisms by which lycopene reduces the risk of metabolic syndrome mainly depend on alleviating oxidative stress and decreasing inflammation [18-22, 126]. Therefore, the effects of lycopene on alleviating oxidative stress and decreasing inflammation may predict the effects of lycopene on metabolic syndrome. With the same level of serum concentrations of lycopene, the effects of serum lycopene on the prevalence of metabolic syndrome are only significant for normal weight or overweight individuals, not for obese individuals.

However, accumulating evidence also supports that lycopene inhibited inflammation and oxidative stress in a dose-dependent manner [38, 39]. Therefore, we hypothesized that highly efficient serum concentration of lycopene may be needed to produce significant effects on participants who are obese. Unfortunately, due to the observational nature of

the study, the serum concentration of lycopene was relatively low among obese participants (0.642 umol/L in the third tertile group) which was also the same as the serum concentration of lycopene among normal weight participants (0.642 umol/L in the third tertile group). In a future observational study, a new analysis method for serum lycopene (for example, relative serum lycopene to the amount of inflammation and oxidative stress in the body) may be used to confirm the hypothesis.

5.1.2. The ratio of serum lycopene to serum triglyceride can predict the health effect of lycopene on metabolic syndrome

Health effects of serum lycopene depend not only on the serum concentration of lycopene, but also on the amount of inflammation and/or oxidative stress in the body; therefore, we hypothesized that the ratio of serum concentration of lycopene to the amount of inflammation and/or oxidative stress could better predict the association between serum lycopene and the health effects. There is no method to directly measure the amount of inflammation and/or oxidative stress in the body. Given the strong associations between oxidative stress [41] and inflammation [42], this study used serum triglyceride to represent the amount of inflammation and/or oxidative stress.

To verify our hypothesis, we examined the association between the ratio of serum concentration of lycopene to serum concentration of triglyceride and the prevalence of metabolic syndrome. As expected, our study showed that higher ratios of serum concentration of lycopene to serum concentration of triglyceride were significantly associated with the reduced prevalence of metabolic syndrome. This result is consistent with findings of previous studies with serum concentration of lycopene as exposure [23-25]. Furthermore, the association between the ratio of serum lycopene to serum triglyceride and the prevalence of metabolic syndrome is significant not only for normal weight and overweight participants, but also for obese participants.

5.1.3. Comparison of two methods for the association between serum lycopene and metabolic syndrome

We compared the associations between metabolic syndrome and lycopene with different measures of serum lycopene: serum concentration of lycopene and the ratio of serum concentration of lycopene to serum concentration of triglyceride. With the serum concentration of lycopene, the association between metabolic syndrome and lycopene was significant only for normal weight and overweight participants, not for obese participants. With the ratio, the association between metabolic syndrome and lycopene was significant not only for normal weight and overweight participants, but also for obese participants. What caused the different results? We hypothesized that the different results may be related to the difference in levels of inflammation and oxidative stress between normal weight/overweight participants and obese participants. Therefore, we compared the mean serum concentrations of lycopene and serum concentrations of triglyceride among all combination of serum levels of lycopene and BMI.

With serum concentration of lycopene (Table 4), the mean serum concentration of lycopene was significantly increased from the first tertile group to the third tertile group for all BMI levels while the mean serum concentration of triglyceride was different among the three BMI levels. The mean serum concentrations of triglyceride was not significantly different between the third tertile group and the first tertile group for normal weight and overweight participants while the mean serum concentration of triglyceride was significantly different between the third tertile group and the first tertile group for obese participants. Therefore, the serum concentrations of lycopene considered to be “high” for individuals with a lower BMI does not produce the same health effects in individuals with a higher BMI because there is more inflammation and oxidative stress production in people with a higher BMI [34, 35].

With the ratio of serum concentration of lycopene to serum concentration of triglyceride (Table 8), the situation is different. The mean serum concentration of lycopene was significantly increased from the first tertile group to the third tertile group for all BMI levels. Also, the mean serum concentration of triglyceride was significantly decreased from the first tertile group to the third tertile group for all BMI levels. Therefore, the ratio method can provide the same conditions (with similar serum concentration of lycopene and serum concentration of triglyceride) for all BMI levels. This may mean that health effects of lycopene are significant not only for normal weight and overweight participants, but also for obese individuals.

In addition, with the serum concentration of lycopene (Table 4), the health effects of lycopene were not different between the second tertile group and third tertile group for normal weight and overweight participants. However, the plateau effect of lycopene may be an alternative explanation—it is possible that the serum lycopene was not high enough in the third tertile group when compared to the second tertile group. With the ratio of serum lycopene to serum triglyceride (Table 8), the health effects of lycopene were significantly different between the second tertile group and third tertile group for all BMI levels because there was a relatively higher concentration of serum lycopene in the third tertile group than in the second tertile group. Therefore, consistent with findings of previous studies [38, 39], this study showed that there is a biologically gradient relationship between the ratio of serum lycopene to serum triglyceride and metabolic syndrome.

The findings of this study indicate that if the relative level of serum lycopene, according to the amount of inflammation and/or oxidative stress, is higher, then serum lycopene may produce significant health effects no matter what the actual serum concentration of lycopene is. This means that a high level of serum lycopene could have significant health effects among adults regardless of their BMI levels.

5.1.4. The health effect of lycopene on mortality among individuals with metabolic syndrome

Serum lycopene can reduce the prevalence of metabolic syndrome among adults. However, whether serum lycopene still has positive health effects among individuals who have developed a health condition with metabolic syndrome is not clear. We hypothesized that serum lycopene can reduce the mortality in individuals with metabolic syndrome.

To test our hypothesis, we examined the health effect of serum lycopene on mortality among NHANES participants with metabolic syndrome. As expected, our study clearly showed that the higher level of serum lycopene was significantly associated with the lower mortality among participants with metabolic syndrome. Therefore, even if individuals who have been classified as having metabolic syndrome, serum lycopene can still help reduce the risk of mortality for them.

5.1.5. The additive health effects of physical activity and lycopene on morbidity of metabolic syndrome

In addition to lycopene, physical activity can reduce the risk of metabolic syndrome [43-45]. However, the effects of physical activity or lycopene alone are insufficient to substantially reduce the risk of metabolic syndrome for overweight and obese individuals [44]. Therefore, exploring the additive effects between physical activity and serum lycopene is important for further reducing the risk of metabolic syndrome among individuals who are overweight and obese.

Our results showed that the additive effects of physical activity and serum lycopene differs by the BMI level. The additive effect was only found among overweight individuals but not for obese individuals. There are two potential explanations for the different effects of physical activity and serum lycopene on the prevalence of metabolic syndrome between

overweight individuals and obese individuals. One explanation is that physical activity reduces the prevalence of metabolic syndrome among obese individuals mainly through other means, such as weight control and an increased level of serum lycopene. Research has shown that physical activity has complex mechanisms on the prevalence of metabolic syndrome, such as controlling weight [162], reducing serum concentration of triglyceride, increasing high-density lipoprotein, improving insulin sensitivity, reducing blood pressure and decreasing inflammation reactions and inducting an anti-inflammatory environment [46-48]. Our results indicated that the increasing the level of serum lycopene may be one of the mechanisms by which physical activity reduces the risk of metabolic syndrome. Consistent with this hypothesis, Cheriyath et al. found that body weight is a more important determinant than physical activity in the development of metabolic syndrome [44]. Therefore, the additive effect of physical activity and serum lycopene among obese individuals may need to be analyzed using more complex conceptual and analytical models that include weight control and serum lycopene change.

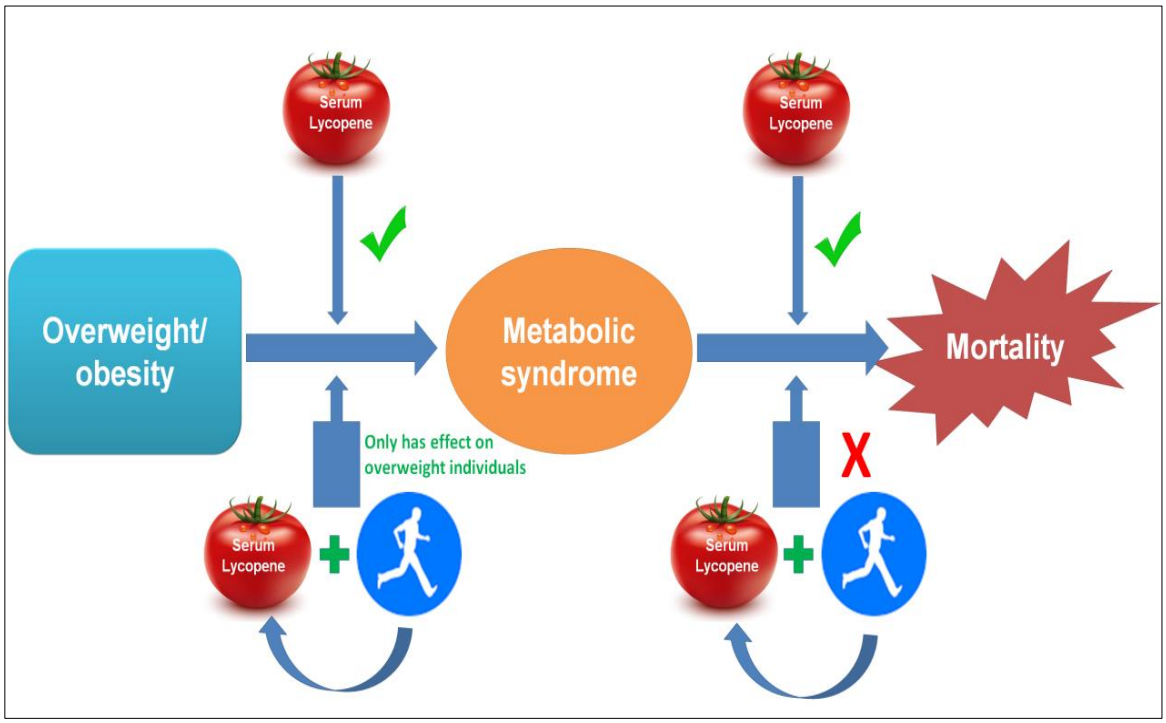
Another explanation is that self-reported physical activity is less accurate among obese individuals than normal weight and overweight individuals. Serum lycopene is an objective variable, while physical activity is a subjective variable in this study. Inaccurate self-reported physical activity will lead to misclassifications. Accumulating evidence supports that obese participants overestimate their vigorous physical activity levels and misclassify the intensity of physical activity than normal weight and overweight participants [163-165]. This means that the misclassification of physical activity is unrelated to classification of metabolic syndrome. As a result, the misclassification of physical activity is a non-differential misclassification, which may have led to results toward the null for obese participants.

5.1.6. The additive health effects of physical activity and lycopene on mortality of metabolic syndrome

For mortality, consistent with the previous findings, physical activity is significantly associated with reduced mortality in patients with metabolic syndrome [69]. Our results also showed that higher serum lycopene levels are significantly associated with reduced mortality in participants with metabolic syndrome. We did not find a significant additive effect of physical activity and serum lycopene on mortality for participants with metabolic syndrome. However, increasing serum levels of lycopene may be one of the mechanisms by which physical activity reduces the risk of mortality among individuals with metabolic syndrome.

In summary, our findings support our conceptual model (Figure 10). Serum lycopene and physical activity can decrease the risk of metabolic syndrome among adults. Serum lycopene and physical activity have an additive effect on metabolic syndrome for overweight individuals, not for obese individuals. Serum lycopene and physical activity can significantly decrease the risk of mortality among individuals with metabolic syndrome. Serum lycopene and physical activity do not have an additive effect on reducing mortality among individuals with metabolic syndrome. However, increased serum lycopene may be one of the mechanisms by which physical activity reduces the risk of morbidity and mortality of metabolic syndrome.

Figure 10. A conceptual model linking lycopene, physical activity, overweight/obesity, metabolic syndrome and mortality



5.2. Strengths and Limitations of the Study

Strengths of this study include a large and representative sample and well-characterized data with many potential risk factors. First, we found that there was a significant interaction effect between serum lycopene and BMI status on metabolic syndrome. Therefore, BMI was an effect modifier of the association between serum lycopene and metabolic syndrome. With effect modification, stratified analysis is an appropriate method to examine the association between exposure and outcome. Therefore, the association between serum lycopene and metabolic syndrome was analyzed separately for normal weight, overweight and obese participants in this study. Secondly, the health effect of serum lycopene on the prevalence of metabolic syndrome may depend not only on serum concentration of lycopene, but also on the amount of inflammation and/or oxidative stress;

therefore, we use the ratio of serum concentration of lycopene to the amount of inflammation and/or oxidative stress as a new measure of lycopene in this study. Our result also supports the idea that the ratio of the serum concentration of lycopene to the amount of inflammation and/or oxidative stress can better predict the association between lycopene and positive health effects than only with serum concentration of lycopene.

Limitations of the study should be also noted. First, as stated previously, a self-report may have produced non-differential and differential misclassification of physical activity levels and other variables. Secondly, although there were strong and dose-response associations between lycopene and the morbidity and mortality of metabolic syndrome, it is not possible to show a causal inference between serum lycopene and metabolic syndrome due to the cross-sectional nature of the study. Thirdly, the effect of serum lycopene may be underestimated in the present study because serum lycopene may be higher in clinical trials when subjects take more fruits and vegetables rich in lycopene or more lycopene supplement than in the current study. Lastly, the effect of changes of serum lycopene on the risk of mortality over time could not be analyzed because of the cross-sectional design of NHANES.

5.3. Conclusion

From a nationally representative sample of the United States adults, our study adds new evidence that the ratio of serum lycopene to serum triglyceride has significant associations with morbidity and mortality of metabolic syndrome. However, due to some limitations of the study, the findings from the study cannot directly provide clinical recommendations, but can provide a new analysis method for future studies between lycopene and health effects.

5.4. Suggestions for the Future Research

Due to being a part of a cross-sectional study, the current study is difficult to build a causal inference between serum lycopene and metabolic syndrome. Further prospective studies with the incidence of metabolic syndrome are needed to confirm these primary results. For example, a cohort of participants may be randomly selected from a community. Then, these participants may be randomly assigned into three groups, a control group, a low-tomato diet and a high-tomato diet. These participants could be followed-up for around 10 years and periodically tested for serum concentration of lycopene and serum concentration of triglyceride and estimated metabolic syndrome condition. Then, we could estimate the causal effect of serum lycopene on the incidence of metabolic syndrome.

According to our findings, even among obese individuals, if the relative level of serum lycopene according to the amount of inflammation and/or oxidative stress is higher, serum lycopene may produce significant health effects no matter what actual serum concentration of lycopene is. Therefore, highly efficient serum concentration of lycopene can produce significant effects on participants who are obese. Thus, future research should test if serum lycopene has a dose-dependent manner on the incidence of metabolic syndrome among obese individuals.

In addition, future research should also determine what the cut-off value of the ratio of serum concentration of lycopene to the amount of inflammation and/or oxidative stress in the body is for substantially reducing the prevalence of metabolic syndrome, separately for normal weight, overweight and obese individuals. This information on the effects of lycopene for individuals with different BMI statuses is expected to be very important for future dosage recommendations.

Although serum triglyceride is strongly associated with inflammation and oxidative stress in the body, serum triglyceride is not a direct measure of inflammation or oxidative

stress. Therefore, more direct measures of inflammation and/or oxidative stress, such as Interleukin (TNF-α, IL-8) and reactive oxygen species (ROS), are needed to estimate the health effects of lycopene based on the ratio of serum concentration of lycopene to the amount of inflammation and oxidative stress.

For the health effect of serum lycopene on mortality among participants with metabolic syndrome, although our findings support the positive health effect of lycopene on mortality for individuals with metabolic syndrome, there are two issues that need to be solved in future research. One issue is the change of serum lycopene during the whole following-up time. Due to the cross-sectional design of NHANES, we do not know if these participants have changed their serum concentration of lycopene during the following-up time. Therefore, a new prospective study for the health effect of lycopene on mortality should have periodic tests of serum concentration of lycopene. Another issue is what causes the reduced mortality among these individuals with a higher level of serum lycopene. In this study, we only know higher serum lycopene has an association with a lower mortality among individuals with metabolic syndrome. Future research can help us to understand what specific disease-related mortality could be reduced in individuals with a higher level of serum lycopene.

For the additive health effect of physical activity and lycopene, serum lycopene is an objective variable; however, physical activity is a self-reported variable which is less accurate among obese individuals than individuals who are normal weight and overweight. Therefore, inaccurate self-reported physical activity will lead to a misclassification error in current study. Therefore, in future research, more objective measurements of physical activity, such as an accelerometer measurement, should verify the additive effect of physical activity and serum lycopene.

In addition, physical activity has complex mechanisms on the morbidity and mortality of metabolic syndrome, such as controlling weight, reducing serum concentration of triglyceride, increasing high-density lipoprotein, improving insulin sensitivity, reducing blood pressure and decreasing inflammation reactions and inducting an anti-inflammatory environment. Therefore, future research should estimate the possible additive effect of physical activity and lycopene on metabolic syndrome and mortality when all these variables are taken into account of in the study.

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