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Percent Body Fat and Fat Distribution are Not Associated with Carotid Artery Intima-media Thickness in Healthy Middle-aged Women

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PERCENT BODY FAT AND FAT DISTRIBUTION ARE NOT ASSOCIATED WITH CAROTID ARTERY INTIMA-MEDIA THICKNESS IN HEALTHY MIDDLE-AGED WOMEN

by

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GRADUATE COMMITTEE APPROVAL

of a thesis submitted by

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This thesis has been read by each member of the following graduate committee and by majority vote has been found to be satisfactory.

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As chair of the candidate's graduate committee, I have read the thesis of Kayleen A. Goff in its final form and have found that (1) its format, citations, and bibliographical style are consistent and acceptable and fulfill university and department style requirements; (2) its illustrative materials including figures, tables, and charts are in place; and (3) the final manuscript is satisfactory to the graduate committee and is ready for submission to the university library.

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ABSTRACT

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Department of Exercise Sciences

Master of Science

Background and Purpose **–** The relationship between abdominal body fat and cardiovascular health is not completely understood. This study investigated the association between percent body fat, fat distribution and intima-media thickness (IMT) in healthy middle-aged women.

Methods **–** 224 middle-aged (mean age = 43.1 years ± 3.0), nonsmoking women were included in this study. The women were assessed with a B-mode, high-resolution ultrasonograph to measure the intima-media thickness of the right common carotid artery (CCA). Measurements for percent body fat and fat distribution were assessed using Bod Pod and waist circumference (WC) measured at the umbilicus, respectively.

Results **–** Data were separated into quartiles with the middle two groups combined in order to identify potential differences in IMT based on waist circumference and body fat percent groups. Mean IMT for the entire sample was .569 mm ± .06. Multiple regression with and without control for potential confounding factors yielded insignificant results for all analyses.

Conclusions **–** In the present study, using a sample of healthy middle-aged women, there were no differences in IMT based on overall body fat percent or waist circumference measurements. This finding is somewhat unexpected, however, regional body fat and CCA-IMT have been shown in some, but not all studies to be positively associated with IMT. More research is needed in this area in order to more clearly identify and understand early risk for cardiovascular disease in women.

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Table of Contents

List of Tables

PERCENT BODY FAT AND FAT DISTRIBUTION ARE NOT ASSOCIATED WITH CAROTID ARTERY INTIMA-MEDIA THICKNESS IN HEATHLY MIDDLE-AGED WOMEN

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Abstract

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Key Words: Intima-media thickness, carotid arteries, body fat

Introduction

The link between body fat and actual cardiac events is not completely understood. Ultrasound imaging over the last several decades has been an effective tool for measuring common carotid artery (CCA) intima-media thickness (IMT) and consequently cardiovascular risk.[1-9] Several studies have compared the relationship between IMT and cardiovascular risk with regional body fat. Regional body fat and CCA-IMT have been shown in some, but not all studies to be positively associated with IMT [10-13]

One study conducted by Ciccoine and associates[13] did find an independent association between WC and CCA-IMT, whereas, Czernichow[14] only found an association after controlling for known coronary artery risk factors. Another study by Lakka[15] did not find any association between WC and CCA-IMT. In these studies there have been differing research methodologies and study populations that may account for the different results. Further research is needed to help determine the relationship between body fat, fat distribution, atherosclerosis and cardiovascular disease risk.

Several studies have examined the relationship between regional body fat and CCA-IMT in low-risk men.[10,14] There are, however, relatively few studies that have specifically focused on low-risk middle-aged women. The present study explored the relationship between percent body fat, fat distribution and CCA-IMT in low-risk, middle-aged women. The subjects included 224 females who were middle-aged and healthy.

Methods

The 224 women were recruited from the general population in several cities of the Mountain West. The women were apparently healthy, mainly Caucasian, and between the ages of 37 and 49 years. Women who planned to become pregnant during the time of the study or who were smokers were excluded from participation.

Anthropometric Measurements

Body composition was assessed using air displacement plethysmography, or the Bod Pod® (Life Measurements Instruments, Concord, CA). At least two measurements for body fat percent were taken on each woman until the results were within one percentage point. These two measurements were then averaged and used to index body fat percentage. Bod Pod has been an established method for assessing body fat percent for more than a decade.[16-18]

Waist circumference was measured with a nonelastic flexible tape measure 0.7 cm wide. During the measurement, subjects wore a one-piece bathing suit and were asked to stand erect with feet together, arms placed comfortably at sides with a relaxed abdomen. They were measured with the tape placed horizontally at the waist across the umbilicus. Subjects were told to breathe normally and the measurement was recorded to the nearest 0.1 cm at the end of a normal expiration.[19]

The tape was completely repositioned after each of the two measurements. The two measurements were averaged and recorded. A test-retest strategy was employed using the subjects of the present study to determine the reliability of the assessment. The intraclass correlation between the two measures was extremely high ($r = 0.999$, $p < 0.0001$).

High-Resolution Carotid Ultrasound and Image Analysis

A SonoSite 180 + Ultrasound portable system (Sonosite Inc., Bothell, WA) was used in conjunction with the SonoCalc software for image acquisition and analysis. The Sonosite 180+ system is a B-mode, high-resolution ultrasonograph, equipped with a 5-MHz transducer. The images were first captured with the 180+ Ultrasound system and then analyzed using SonoCalc software. The SonoCalc software is automatically calibrated for SonoSite 180+ Ultrasound images.

The SonoCalc automated software analysis program used in this study was described and validated by Fritz and associates.[1] All CCA images were taken from the right common carotid artery. The participants were asked to lie in the supine position during the ultrasound procedure. Each image of the right common carotid artery was captured longitudinally by a technician trained in carotid ultrasound imaging. The CCA images of all participants were taken in multiple directions to ensure quality imaging of the entire arterial segment. The images were captured at a point 1 cm proximal to the common carotid bifurcation. The images were labeled and permanently saved as a bit map file for analysis.

Clinical and Metabolic Assessment

To help control for the effect of possible confounding influences, other variables were considered in the final analyses. These variables included: total cholesterol, LDL cholesterol, HDL cholesterol, fasting blood glucose levels, systolic and diastolic blood pressure, and physical activity.

Total cholesterol, HDL-C, LDL-C, and blood glucose were calculated using a certified hospital laboratory. Blood samples were taken from the antecubetal vein after the participants fasted for at least 12 hours.

Blood pressure was taken with a standard mercury sphygmomanometer. Each subject was measured while sitting after 15 minutes at rest. The average of three measurements was recorded at two different times one week apart.

Activity levels were assessed using the ActiGraph accelerometer (formerly, CSA) (Pensacola, FL). The accelerometers measured daily physical activity of participants. Subjects were required to wear the accelerometer using a belt placed at the left hip for seven consecutive days. The subjects were instructed not to remove the accelerometer from the hip except for activities involving submersion in water or loosening the belt for sleeping.

A trained researcher analyzed all images using the SonoCalc software to determine the average IMT of the captured images for the 224 women. The images were analyzed by a different person than the ultrasound technician who originally captured the images in order to reduce the likelihood for possible measurement bias.

The researcher identified suitable images based on clarity from a total of 23,768 images. Within the subset of clear images, a total of nine images were chosen to determine an average IMT for each participant. The nine images were chosen by randomly selecting three images from three different angles of the CCA. These angles included the anterior view, direct view, and posterior view of the right common carotid artery. A 1 cm IMT segment was measured to the nearest 1/1000 mm at the point 1 cm proximal to the common carotid bulb using the SonoCalc software. The nine measurements were combined and averaged to determine a mean IMT for each subject.

In six subjects, there were certain instances among the nine measurements taken for a mean IMT, where the carotid segment was unclear and the software was unable to identify the intima/lumen surface. In these instances, a manual analysis was performed using the same process described by Fritz et al.[1] All images included in the study were analyzed at the far wall.

Statistical Analysis

Statistical analysis was performed using SAS software, version 9.1 (SAS Institute, Inc., Cary, NC). Multiple regression was used to determine the strength of the relationship between the criterion and the predictor variables. Women were separated into quartiles with the middle two groups combined to compare potential differences in IMT for waist circumference and body fat distribution. Controlled variables included activity level, age, cholesterol, fasting blood glucose and blood pressure. For all of the tests, a $p < 0.05$ was used to determine statistical significance.

Results

Subjects

Table 1 depicts descriptive characteristics of subjects. Subjects ranged in age from 37 years to 49 years, had normal, if not low average systolic and diastolic blood pressure, 107 ± 10 mm Hg and 71 ± 8 mm Hg, respectively, and had desirable average total cholesterol levels below 200 mg/dl (mean = 186 mg/dl, SD = 30 mg/dl). The mean LDL levels were near optimal 109 ± 27 mg/dl and the mean HDL levels were normal 57 ± 13 mg/dl. The subjects also had normal glucose levels with a mean fasting glucose level of 86 ± 6 mg/dl. The mean waist circumference was 82 ± 10 cm and mean body fat percent was 32 ± 7.

Comparison of Body Fat % Groups and CCA-IMT

Analyses indicated no significant correlation between body fat percentage and CCA-IMT ($r^2 = 0.002$). For multiple regression analyses, subjects were grouped into quartiles according to body fat percentage. The middle two groups were then collapsed to facilitate comparison of CCA-IMT for those with low, moderate, and high levels of body fat percentage. Again, no significant differences were found in CCA-IMT between the body fat percentage groups. Even after controlling for potential confounding factors known to be related to CVD risk, such as blood pressure, fasting glucose, cholesterol, and activity level, all comparisons for differences in CCA-IMT by body fat percentage were nonsignificant (see Table 2).

Comparison of Waist Circumference Groups and CCA-IMT

As was found with body fat percentage analyses, no significant correlation existed between waist circumference and CCA-IMT ($r^2 = 0.013$). Subjects were also grouped into waist circumference quartiles with the middle two groups collapsed. No significant differences were found in CCA-IMT among the waist circumference groups. Even after controlling for the same potential confounding factors used in the body fat percentage analyses, all comparisons for differences in CCA-IMT by waist circumference were nonsignificant (see Table 3).

Other Findings

Age was found to be positively correlated to CCA-IMT. Even though the range of the subject's age was narrowly limited to only 12 years, there was a significant correlation between age and IMT ($P < 0.02$). Systolic ($P < 0.0003$) and diastolic blood pressure ($P < 0.02$) were also significantly associated with IMT.

Discussion

The primary purpose of this study was to investigate the potential link between body fat distribution, percent body fat and CCA-IMT. This study was a cross sectional analyses of middle-aged, mostly Caucasian women, who had low CVD risk. The findings of this study indicate that, in healthy middle-aged women, there is no apparent relationship between total percent body fat or fat distribution and CCA-IMT. Multiple regression analyses also showed no significant differences in CCA-IMT for either body fat percentage groups or waist circumference groups, even while controlling individually and collectively for a number of cardiovascular risk factors, including age, cholesterol, blood pressure, fasting glucose, and physical activity level.

One possible reason there was no relationship found between CCA-IMT and percent body fat and fat distribution, even after controlling for CVD risk factors, is that the women in the present study population were recruited from a geographic area that has the second lowest annual death rates for coronary heart disease in the United States (90.4/100,000).[20] The region is also known to have the lowest blood pressure prevalence rates for adults over 18 years in the US (18.4%) and the lowest prevalence of current smokers (9.8*%).*[21] In short, the current sample had several characteristics indicating low

CVD risk. Apparently, there is little association between abdominal fat and total body fat and IMT in low-risk middle-aged women.

The findings of the present study are similar to a study conducted in France by Tremollieres and colleges.[12] This study involved 906 healthy Caucasian early postmenopausal women aged 45 to 65 years. Subjects were measured for IMT by ultrasound and body composition using dual energy x-ray absorptiometry (DXA). The effect of other potential confounders related to coronary risk were also assessed. The mean IMT collectively for the subjects was 0.520 (± 0.07). The results of the study indicated that only age, systolic and diastolic blood pressure were independently and significantly correlated with IMT. The researchers also concluded that a possible reason for no other significant findings in their study was the low risk of coronary heart disease in the women.

This study was not without limitations. The subjects were women of similar age, mainly Caucasian and from the same geographic area resulting in minimal diversity in race and environment. The subjects were also relatively healthy and were free from diagnosed cardiovascular disease.

The findings of the current research may have been different for obese and/or older more racially diverse women. However, it is interesting to note that body fat ranged from approximately 12% to 50% and waist circumference ranged from approximately 62 cm to 117 cm.

The current study does, however, support existing research suggesting that age is positively correlated to CCA-IMT.[22,23] The subject’s age was narrowly limited to a little over a decade and there was a significant correlation between age and IMT. Homma and colleges[23] found that mean IMT has a tendency to increase linearly with age for all decades of life. This change is demonstrated by an increased CCA-IMT mean of 0.48 mm at 40 years old to 1.02 mm at 100 years old. The predicted IMT for women in the present study using the Homma equation is 0.504 mm while the actual mean for the sample was 0.569 mm.

Not only was age significantly associated with IMT, but systolic and diastolic blood pressures were also significantly associated with IMT, respectively. As blood pressure increased, mean IMT increased. Other studies have found similar associations with systolic blood pressure[13,24] or both systolic and diastolic blood pressure.[12] Another study by Lakka and associates[25] found that even mild increases in systolic blood pressure may accelerate the progression of preclinical atherosclerosis.

Even though the current study did not find an association between WC and IMT other studies have found WC and body composition to be positively correlated with IMT. Czernichow and associates[14] investigated the role body composition and fat repartition have in relationship to the large arteries in the body. There were 1014 men and women who participated in the study. Fat mass and fat-free mass were measured using bioimpedance. WC and WHR were measured to determine fat repartition and ultrasound was used to determine IMT of the CCA. After adjusting for major known CAD risk factors, fat-free mass, fat mass, and WC were all positively correlated to CCA IMT ($p < 0.05$).

A study by Takami and associates[10] found an association when comparing intra-abdominal fat verses general adiposity and IMT. The cross sectional study included 849 Japanese men aged 50.3 ± 8.5 years (range 20-78 years). The results of the study indicated that BMI, WC, and WHR were positively correlated with IMT however, when adjusted for BMI, the association between waist circumference and carotid IMT was eliminated. The subjects' mean IMT in this study was 0.700 ± 0.130.

Another study conducted in Italy by Mario De Michele and associates[11] involved a sub-sample of middle-aged women and examined the relationship between obesity, central fat distribution and IMT. The sub-sample included 310 women who were being investigated for possible causes of CVD and cancer. The sub-sample of women was measured for IMT and lumen diameter of the CCA by B-mode ultrasound. WHR and BMI were also measured.

The results of the study indicated that over time, a gradual increase in the IMT of the CCA was noted in lean subjects (0.94 ± 0.01 millimeters (mm)) when compared with overweight (0.98 ± 0.01 mm) and obese women (1.02 ± 0.02 mm) in the study. Women with higher WHR (> 0.85 cm) had thicker IMT (1.02 ± 0.01 mm) when compared to those who had smaller WHR (WHR < 0.81, IMT = 0.94 ± 0.01 mm; WHR 0.81-0.85, IMT = 0.98 ± 0.01 mm, $p < 0.01$) values. BMI ($R^2 = .30$, $p < 0.003$) and WHR ($R^2 = .28$ $p < 0.006$) were shown to be significant predictors of IMT in multivariate analyses, independent of traditional and nontraditional cardiovascular risk factors including age, blood pressure, lipid abnormalities, and fasting insulin values.

A recent meta-analyses conducted by Lorenz and colleges[26] investigated an increasing IMT and the prediction of cardiovascular events. The analyses reviewed data from 37,197 subjects who were followed for a mean of 5.5 years. The findings of the study suggest that the absolute IMT difference of 0.1 mm increases the risk of a myocardial infarction by 10% to 15%, and stroke increases as much as 13% to 18%. According to Lorenz's study, the women in the current study have a lower risk of a myocardial infarction and stroke with a mean IMT of 0.569 ± 0.061 mm in comparison to Mario de Michele's study with an IMT range of 0.94-1.02 mm.

Conclusion

This study found that percent body fat and fat distribution are not significantly correlated to CCA-IMT in healthy middle-aged women. Other researchers have found positive associations between IMT and WC in other populations with a higher mean IMT and higher risk subjects, or when using other techniques to measure body composition or regional fat. More research is needed in this area to determine the extent to which low-risk middle-aged women may be at risk because of body composition and fat distribution.

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Table 1. Descriptive Statistics of Subjects

	N*	Mean	SD
IMT (mm)	224	0.569	0.061
Body Fat (%)	225	32.2	7.3
Waist Circumference (cm)	224	81.9	10.4
Age (y)	225	43.1	3.0
Activity Counts (x .001)	221	2620	9455
Cholesterol (mg/dl)	208	185.6	30.2
LDL-Cholesterol (mg/dl)	208	108.6	26.6
HDL-Cholesterol (mg/dl)	208	56.6	12.9
Fasting Glucose (mg/dl)	205	86.4	6.3
Systolic BP (mm Hg)	225	106.9	10.1
Diastolic BP (mm Hg)	225	70.5	7.5

*Total N varies for certain descriptive measures because all participants did not have complete data sets

Table 2. Multiple Regression Analysis with CCA-IMT and Body Fat

	Body Fat % Groups				
	Low	Moderate	High	F	*P*
	Mean (SD)	Mean (SD)	Mean (SD)		
IMT	0.565 (0.056)	0.571 (0.065)	0.567 (0.059)	0.22	0.81
Variable Controlled					
Waist Circumference	0.572	0.572	0.558	0.67	0.51
Age	0.569	0.571	0.565	0.16	0.85
Activity Counts	0.561	0.573	0.568	0.63	0.53
Cholesterol	0.568	0.572	0.565	0.22	0.80
LDL-Cholesterol	0.569	0.572	0.565	0.18	0.83
HDL-Cholesterol	0.565	0.572	0.569	0.21	0.81
Fasting Glucose	0.568	0.570	0.566	0.08	0.93
Systolic BP	0.568	0.572	0.563	0.41	0.66
Diastolic BP	0.567	0.572	0.564	0.37	0.69
All of the Above	0.573	0.572	0.558	0.56	0.57

*Means for control variables are shown as adjusted means without standard deviation

Table 3. Multiple Regression Analysis with CCA-IMT and WC

	Waist Circumference Groups				
	Low	Moderate	High	F	*P*
	Mean (SD)	Mean (SD)	Mean (SD)		
IMT	0.557 (0.058)	0.574 (0.060)	0.571 (0.066)	1.49	0.23
Variable Controlled					
Body Fat %	0.551	0.574	0.577	2.02	0.14
Age	0.558	0.574	0.570	1.16	0.31
Activity Counts	0.555	0.573	0.573	1.69	0.19
Cholesterol	0.560	0.573	0.571	0.75	0.47
LDL-Cholesterol	0.561	0.573	0.571	0.61	0.55
HDL-Cholesterol	0.555	0.573	0.575	1.49	0.23
Fasting Glucose	0.560	0.573	0.568	0.76	0.47
Systolic BP	0.563	0.573	0.566	0.69	0.50
Diastolic BP	0.560	0.574	0.568	0.94	0.39
All of the Above	0.559	0.572	0.570	0.62	0.54

*Means for control variables are shown as adjusted means without standard deviation

Appendix A

Prospectus

Chapter 1

Introduction

In 2004, the prevalence of coronary heart disease in the United States was almost 16 million persons. Of these 16 million people 7.2 million were women. Additionally, there were 700,000 people who suffered a stroke in 2004, and 373,000 of them were women (1). In years past many women did not think that heart disease and stroke were significant risk factors. Women are becoming increasingly aware of the risk of cardiovascular disease (CVD) and stroke as more research is being done to determine the potential risks of disease and death (2).

Atherosclerotic burden in the arteries is a risk factor that contributes to CVD (3). By screening for increased intima-media thickness in the common carotid artery (CCA) clinicians can identify potential risk for heart disease and stroke (4-8). Increased body fat (9) and the distribution of fat in the body are also contributing factors for CVD (10,11), especially excess abdominal fat (12,13). The relationship between abdominal body fat and cardiovascular health is not completely understood (11,14-16). By evaluating the arterial wall thickness in the common carotid artery (CCA) and the amount and location of fat in the body, researchers may more fully understand predisposing risk factors for cardiovascular events for both men and women.

Over the past several decades, researchers have studied the associations of fat distribution, percent body fat, and cardiovascular risk (12,14,17-28). Waist circumference (WC) and waist-to-hip ratio (WHR) are commonly used in research studies to account for body shape and size. These assessments are typically made around the widest part of the abdomen and hip areas of the body. The WHR is then a ratio between the waist and hip measurements. In the past, researchers found that as waist-to-hip ratio increases so does risk for coronary heart disease (29).

In one prospective study involving 700 male and female subjects, WC and HC had an independent and opposite effect on cardiovascular risk (30). Results of the study indicate that a narrow waist and large hip circumference may aid in the protection against the risk of cardiovascular disease. Researchers from this study concluded that WHR may not be the best indicator for cardiovascular risk. Some researchers theorize that the hip circumference yields a structurally different muscle, bone, and fat mass measurement for the hips in the gluteal area than does the abdomen and therefore could explain differing outcomes (30,31). Therefore, WC may be a better indicator of cardiovascular risk than WHR (32).

Measurement of the intima-media thickness (IMT) of the CCA is also a valid and reliable measure for determining atherosclerotic burden (33-39). Atherosclerotic buildup is measured using diagnostic ultrasound by assessing the thickness of the intima and media layers of the common carotid artery and the echogenic material located in the arterial lumen (7,34,40). Ultrasound imaging and associated analytical software can be used to detect the amount of plaque and any minute changes in plaque and IMT that occur (35,36,41,42). If atherosclerotic plaque is found, patients can begin a treatment program to diminish the existing plaque, thus reducing the risk of disease or death (43-45).

Several studies have compared the relationship between IMT and cardiovascular risk with waist circumference and waist-to-hip ratio. WHR and WC have been shown in some, but not all studies, to be positively correlated to IMT (14,15,26,30,46). In these studies there have been differing research methodologies that may account for the different results. Further research is needed to help determine the relationship between body fat and fat distribution, and atherosclerosis and cardiovascular disease risk.

In spite of increased awareness, knowledge, lifestyle changes, medical technology, and advancing medicines, heart disease and stroke remain among the top five leading causes of death for women in the United States (47). By studying the relationship between IMT and body fat distribution researchers and clinicians may be able to more effectively understand the risks for future cardiovascular events (11,13). The purpose of this study is to assess the relationship between the CCA IMT and body fat and fat distribution in middle-aged women.

Statement of the Problem

The purpose of the proposed study is to evaluate intima-media thickness of the CCA in relationship to percent body fat and fat distribution in premenopausal middle-aged women. Currently, there is little evidence regarding the correlation between IMT and body composition as assessed by dual-energy x-ray absorptiometry (DEXA) and WC. This study will investigate the relationships between body fat, fat distribution and IMT. If a positive relationship exists between IMT and percent body fat and fat distribution in women this study may have clinical significance. Findings from this study may potentially help clinicians improve assessment techniques for risk of cardiovascular disease and stroke and aid in prevention practices.

Research Questions

1. To what extent does percent body fat correlate with common carotid artery intima-media thickness?
2. To what extent does regional fat distribution correlate with common carotid artery intima-media thickness?

Assumptions

The following assumptions apply to this study:

1. The experience and training of the researchers is adequate to accurately evaluate intima-media thickness of the common carotid artery.
2. The screening administrator for the ultrasound protocol is trained sufficiently to administer the protocol accurately and reliably.

Limitations

1. Arterial measurement of subjects will be limited to the right common carotid artery.
2. Other factors which may not have been controlled for can affect the outcome of intima-media thickness of the carotid artery.

Delimitations

This research will be conducted in collaboration with the Brigham Young University Lifestyle Project. The study will include only data gathered from the third phase of the cohort where IMT of the CCA was measured. The subjects included females who were presumed to be healthy, premenopausal, and non-obese (BMI < 30). The ages of the women were between 37-49 years. At the time of data collection, all subjects were non smokers, most were Caucasian, educated, married, and none were pregnant or planned to become pregnant during the course of the study.

Operational Definitions

B-Mode – Brightness Mode – Is used in ultrasonic measurements to display a two-dimensional image.

BMI – Body Mass Index is a ratio of the height of an individual measured in meters divided by weight that is squared in kilograms.

M-Mode – Motion Mode is a technique used in ultrasonic imaging to study the movement of internal body structures.

SonoCalc – Software that is compatible with the SonoSite 180+ Ultrasound System used to measure IMT.

SonoSite 180+ Ultrasound System – A portable ultrasonography system that has the ability to capture images of the CCA.

WHR – Waist-to-hip ratio is a ratio measured in centimeters by dividing the waist circumference by hip circumference.

Chapter 2

Review of Literature

Noninvasive ultrasonography is used for a variety of health evaluations. Some of these evaluations involve the premature signs of atherogenesis, including impaired endothelial function, increased arterial wall thickness, and atherosclerotic lesions (48). In postmortem examinations subjects that died suddenly from coronary artery disease also had a high prevalence of atherosclerosis in the coronary arteries and in other vascular beds (5). The earliest indications of atherosclerotic lesions most often occur first in the aorta, then the coronary and carotid vessels (48). This review of literature will explore the research related to the relationship of 1) percent body fat, body fat distribution and intima-media thickness of the carotid artery, 2) coronary heart disease and IMT, and 3) the reliability of ultrasound.

Body Fat, Fat Distribution and Intima-Media Thickness of the Carotid Artery

Takami et al. (14) conducted a study comparing intra-abdominal fat verses general adiposity on metabolic abnormalities and the risk of atherosclerosis. The cross sectional study involved 849 Japanese men aged 50.3 ± 8.5 years (range 20-78 years). Measurements of IMT were obtained using B-mode ultrasound. General adiposity was determined using height and weight measurements to determine BMI (kg/m^2). The abdominal subcutaneous fat (ASF) and intra-abdominal fat (IAF) were measured by computed tomography (CT) scans. WC and WHR were used as an additional method for abdominal fat measures. Controls for confounding factors such as glucose tolerance, insulin resistance, and serum lipids levels were also considered in the analyses.

The results of the study indicate that BMI ($r = 0.138$, $p = 0.0001$), WC ($r = 0.126$, $p = 0.0005$), WHR ($r = 0.165$, $p = 0.0001$), IAF ($r = 0.098$, $p = 0.0058$), and ASF ($r = 0.104$, $p = 0.0034$) were positively correlated with IMT. However, when adjusted for BMI the association of IAF, ASF, and waist circumference with carotid IMT is eliminated. When BMI is controlled, WHR continues to be significantly correlated with IMT ($r = 0.106$, $p = 0.0034$). After further control for IAF, BMI is significantly correlated with IMT ($r = 0.107$, $p = 0.0030$).

Czernichow et al. (15) examined 1014 participants in a study to determine the role body composition and fat repartition have in relationship to the large arteries in the body. This study included 504 men and 510 women who were middle aged. Fat mass and fat-free mass were measured using bioimpedance. WC and WHR were measured to determine fat repartition and ultrasound was used to determine IMT of the CCA. After adjusting for major known CAD risk factors, fat-free mass, fat mass, and WC were all positively correlated to CCA IMT and lumen diameter ($p < 0.05$).

Another study conducted in Naples, Italy by Mario De Michele and associates (26) involved a sub-sample of middle-aged women and examined the relationship between obesity, central fat distribution and IMT. The sub-sample included 310 women from a larger on-going study of 5,000 women who were being investigated for possible causes of CVD and cancer in women. The sub-sample of women were measured for IMT and lumen diameter of the CCA by B-mode ultrasound. WHR and BMI were also measured.

The results of the study indicate that over time, a gradual increase in the IMT of the CCA was noted in lean subjects (0.94 ± 0.01 millimeters (mm)) when compared with overweight (0.98 ± 0.01 mm) and obese women (1.02 ± 0.02 mm) in the study. Also, women with higher WHR (> 0.85 cm) had thicker IMT (1.02 ± 0.01 mm) when compared to those who had smaller WHR (WHR < 0.81 cm, IMT = 0.94 ± 0.01 mm; WHR 0.81-0.85 cm, IMT = 0.98 ± 0.01 mm, $p < 0.01$) values. BMI ($R^2 = 30\%$, $p < 0.003$) and WHR ($R^2 = 28\%$, $p < 0.006$) were shown to be significant predictors of IMT in multivariate analyses, independent of traditional and nontraditional cardiovascular risk factors including age, blood pressure, lipid abnormalities, and fasting insulin values.

Another study (11) used data from boys and girls participating in the on-going Amsterdam Growth and Health Longitudinal Study (AGAHLS) to determine the relationship between body fatness, fat distribution, carotid atherosclerosis and arterial stiffness. At the beginning of the study, subjects were boys and girls with a mean age of 13.1 (± 0.8) years. Several years later subjects were tested again at a mean age of 36.5 (± 0.6). The latter measurement was taken with 161 men and 175 women using skin fold thickness, WC, WHR, and non-invasive ultrasound imaging for the IMT of the right CCA.

The results of the study indicate that abdominal, truncal, and abdominal subcutaneous fat are independently associated with arterial stiffness. Skinfold thickness of the boys during adolescence compared with IMT of the CCA at age 36 years imply that the amount of total and central subcutaneous fat at an early age can influence carotid wall thickening later on in life, thus increasing the risk of atherosclerosis ($p < 0.05$).

Bonora et al. (46) compared IMT of the CA in nondiabetic and non-insulin dependent diabetes mellitus (NIDDM) patients to determine differences in early atherosclerosis risk and other cardiovascular risk factors. The study included 58 nondiabetic and 56 NIDDM patients who were selected at random from an outpatient diabetes clinic or a clinic of internal medicine. Measurements for IMT, BMI, WHR, blood pressure, glycohemoglobin, and fasting plasma glucose, serum lipids, and insulin were taken on all subjects. The results of the IMT were determined as significantly higher in diabetic patients (1.44 ± 0.15 mm, $p < 0.001$) than nondiabetic subjects (1.19 ± 0.15 mm, $p < 0.001$). This significance persisted even after adjusting for sex, age, BMI, WHR, hypertension, dyslipidemia, and smoking status (diabetic = 1.39 mm vs. nondiabetic = 1.24 mm, common SD 0.12, $p < 0.001$). In multiple regression analyses nondiabetic patients' IMT was independently associated with age and WHR ($t = 4.09$, $p < 0.001$).

Yamamoto and associates (49) conducted a study to assess the association between intra-abdominal fat and carotid atherosclerosis. This study involved ninety-eight non-obese men, aged 40-67 years. IMT and visceral fat were both measured using ultrasound. The IMT was measured in bilateral carotid arteries using the average of the thickness area and 1 cm upstream and 1 cm downstream from the thickest point. Fat distribution was measured with ultrasound scanning along the linea alba from the xiphoid process to the navel. Researchers concluded from the results that abdominal wall fat index (AFI) was significantly correlated with IMT ($r = 0.23$, $p < 0.05$) and an even greater correlation was shown between IMT and preperitoneal fat ($r = 0.37$, $p < 0.005$).

Lawlor et al. (50) conducted a study in an effort to determine differences in body fat distribution and carotid intima-media thickness in men and women. The researchers used some of the original male participants of the British Regional Heart Study and also involved a newly randomly selected group of women from the same type of population, for a total of 800 men and women. The subjects were between the ages of 56-75 years. Results of the study indicate that IMT and WHR have a continuous linear association in both men and women. The similarity of this association between WHR and IMT was identical in both genders. After age adjusted analyses, there was a 14% increase of IMT in men compared to women (geometric means for male to female age adjusted ratio 1.14; 95% confidence interval 1.07 to 1.21).

Coronary Heart Disease and Ultrasound

The Atherosclerosis Risk in Communities Study (ARIC) (51) used ultrasonographic measurements for IMT measurements of the carotid artery and popliteal artery. The cohort study involved 13,870 black men and women aged 45-64 years old. The results of the study indicate that there is an association between IMT and CAD in middle-aged adults aged 45-64 years old. Subjects with a history of myocardial infarction had a mean IMT that was greater than subjects without the disease. This was the same among all the subjects regardless of race or gender. Similar results were observed for IMT and angina (0.04 mm), cerebrovascular disease (0.05 mm), peripheral vascular disease (0.15 mm), diabetes (0.06 mm), and all CVD (0.06 mm).

From the ARIC study (52), a case control study emerged using 772 selected persons from the sample population. In the case control study, the participants were evaluated based on carotid arterial wall thickness and CHD incidence. The results of the study indicate that those with a mean IMT of 1 mm or more were more likely to have CHD than those with lower mean IMT scores ($P < 0.0001$). Based on findings from this study, researchers concluded that mean carotid IMT is a good predictor of the future incidence of CHD.

The Angina Prognosis Study in Stockholm (APSIS) (53) evaluated 558 men and women with stable angina pectoris who had a mean age of 60 ± 7 years. The subjects were assessed for IMT plaque in the carotid and femoral arteries and lumen diameter. The results indicate that the carotid plaques were related to the risk of myocardial infractions and risk of cardiovascular death ($P = 0.056$).

Reliability of Ultrasound

In many studies carotid 2-dimensional ultrasound imagining has been shown to be a valid and reliable assessment tool for imagining the extracranial carotid arteries and wall thickness. The Asymptomatic Carotid Artery Progression Study (ACAPS) (37) involved 919 randomized participants, aged 40-79 years old, with various coronary risk factors. The study utilized a B-mode ultrasound system. The IMT measurements were taken from subjects at the walls of three arterial segments of both the left and right arteries. Through post hoc calculations the study indicated 90% power to detect changes in IMT < 0.02 mm/y. The ACAPS measurement protocol provided highly reliable serial IMT data.

Kazmierski et al. (38) evaluated the reproducibility of high-resolution analog ultrasonographic measurements of the IMT in the CA. The study involved 25 subjects, 15 of whom were post-stroke victims and 10 healthy individuals. Three observers with different levels of experience and skills in ultrasonography performed the same measurements twice with at least 3 weeks between sessions. The results of the study indicated that the three observers demonstrated a reliable and reproducible measurement of the CA IMT. Intraobserver variability was demonstrated with a correlation coefficient from 0.92 to 0.95 ($p < 0.0001$).

In a review of literature by Kanters et al. (39), researchers focused on the intraobserver and/or interobserver variability for different methods of measuring IMT using ultrasound imaging. Before this review it was difficult to compare different studies involving two-dimensional B-mode ultrasound because of the varying methods for image attainment and IMT analysis. The findings contained in this review, concluded that the variability of IMT is lowest and the reproducibility is best when the mean of the IMT is calculated from multiple images that consist of more than one direction in the CCA.

Baldassarre et al. (33) conducted a cross sectional study that involved intraobserver and interobserver variability of IMT. The 963 participants underwent measurements performed manually with electronic calipers in real time with B-mode ultrasound imaging. The results indicate similar reproducibility results for quantitative processing of frozen images (39). The coefficients of variation for intra-observer variability measurements of the mean maximum IMT was 4.2% ($r = 0.95$). The coefficient of variation for inter-observer variability was 7.3% ($r = 0.96$).

Schulte-Altedorneburg and colleges (34) examined the correlation between in vivo ultrasound of the IMT, lumen diameter, and the cross-sectional area of the CCA.

Subjects' data were collected using gross pathology and histology. Sixty-six critically ill neurological patients with a mean age of 71 ± 13.2 years (range 37 to 95 years) were included in this study. There were 37 men and 29 women who received B-mode ultrasound measurements of the CCA a few days prior to death. After death during autopsy examinations the carotid specimens were removed from the subjects. The results of the study indicate that transcutaneous B-mode ultrasound is a reliable in vivo measurement of the cross-sectional area of the CCA ($R^2 = 0.497$) and lumen diameter ($R^2 = 0.389$) when compared with macroscopic postmortem analysis.

Fritz et al. (35) conducted a study to determine if an automated IMT software analyses system was as precise and accurate as the standard manually controlled electronic calipers. Sonographers who were experienced in general cardiovascular imaging, but without extensive experience in capturing the image and measurement of the IMT, were included in this study. Subjects included 30 participants, involving men and women. Three sonographers measured 1 centimeter (cm) segments in the CCA from the far and near walls using 120 images with both the manual electronic calipers and with SonoCalc software on each subject. Each segment was measured twice. The results of the study indicate that the differences in the measurements using the SonoCalc and manual calipers were insignificant. In addition, reproducibility using SonoCalc was more significant than the manual calipers among the three sonographers ($p < 0.0001$).

Most studies in the past were conducted with analog systems, however, as technology increases clinical trials may need to consider the new digital ultrasound systems. One particular study by Baldassarre and colleges (36) involved the comparison of both the analog and digital systems. The study involved 22 healthy subjects that had 4 replicate scans: two with the analog system and two scans with the digital system. The Biosound II system and the Esaote AU4 system were used in this study. The results of the study indicate that even though the analog system provides high quality results ($r = 0.97$), the digital system provides greater reproducibility ($r = 0.99$).

Summary

Detection of atherosclerotic plaque and IMT measurements assessed by ultrasound has been used in recent years. The ultrasonographic methods of measurement can assess less reachable vessels such as the coronary and cerebral arterial systems (5). Determining the thickness of the intima-media layer can alert clinicians to begin preventive measures with patients who are asymptotic or in the beginning stages of risk for cardiovascular disease and atherosclerosis (54). Ultrasound measurements of the CCA can be conducted with safety, precision, and in a reproducible manner (5). In addition, WC can be safely used to accurately assess fat distribution in conjunction with the ultrasound to determine possible correlations for cardiovascular risk factors in men and women.

Chapter 3

Methods

Subjects

This research will be conducted in collaboration with the ongoing Brigham Young University Lifestyle Project. The original prospective cohort study included 272 middle-aged women. The women were initially recruited from the general population in Utah County. The women were presumed to be healthy, premenopausal, non-obese (BMI < 30), and between the ages of 37 and 49 years and most were Caucasian. Any women who planned to become pregnant during the time of the study or who were smokers were excluded from participation.

Researchers have continued to gather data periodically on the participants since 1998, only during the second phase of the study, each participant received ultrasound screening for IMT in addition to the assessment for body composition with DEXA and waist circumference. The proposed project will only include data gathered during the third phase of collection. There were approximately 200 women involved in the study at this point in time. The women had an average age of 37-49 years. The study will be approved through the Institutional Review Board at BYU.

Anthropometric Measurements

During the third phase of data collection in 2002, body composition was assessed using Hologic's dual-energy x-ray absorptiometry machine (model 4500 W, Hologic QDR, Waltham, MA). DEXA is a valid and reliable measurement for determining body composition (55,56). Each participant was asked not to eat four hours before the assessment and to rid the body of any waste directly before the procedure began. Each participant wore a one-piece BYU issued bathing suit during the time of assessment. The subjects were first weighed using an electronic scale (Tanita Corporation, Japan) and then immediately scanned with DEXA. Full body scans for each participant were taken from licensed technicians. The images were then analyzed using QDR 11.2 software.

Waist circumference was measured with a non-elastic flexible tape measure 0.7 cm wide. During the measurement subjects were wearing a one piece bathing suit and asked to stand erect with feet together, arms placed comfortably at sides with a relaxed abdomen. The observer stood in front of the subjects and measured the waist by placing the tape measure horizontally across the umbilicus. Subjects were told to breathe normally and the measurement was recorded at the end of a normal expiration. The waist circumference of the subjects was determined by measuring to the nearest 0.1 cm.

High-Resolution Carotid Ultrasound

A SonoSite 180 + TITAN Ultrasound portable system was used in conjunction with the SonoCalc software for image acquisition and analysis. The Sonosite 180+ system is an M-mode, high-resolution ultrasonograph, equipped with a 5-MHz traducer. The images were first captured with the 180+ Ultrasound system and will be analyzed using SonoCalc software. The SonoCalc software is automatically calibrated for SonoSite 180+ Ultrasound images.

High-Resolution Carotid Ultrasound Imaging

The SonoCalc automated software analysis program used in this study was described and validated by Fritz et al. (35). All CCA images were taken from the right common carotid artery using a Sonosite 180+ Ultrasound system (Sonosite Inc., Bothell, WA). The participants were asked to lie in the supine position during the ultrasound procedure. Each image of the right common carotid artery was captured longitudinally by a technician trained in carotid ultrasound imaging. The CCA images of all participants were captured in multiple directions to ensure the best quality imaging of the entire arterial segment. Approximately 120 images were captured for each person. The images were captured at a point 1 cm proximal to the common carotid bifurcation. The images were labeled and permanently saved as a bit map file for later analysis.

Design

For this study a trained researcher will analyze all images using the SonoCalc software to determine the average IMT of the captured images for the approximate 200 women involved in the second phase of the original cohort. The images will be analyzed by a different person than the ultrasound technician who originally captured the images in order to reduce the likelihood for possible measurement bias.

The researcher will determine suitable images from the total 23,768 images previously captured and place them in a subset of images based on clarity. Within the subset of clear images a total of nine images will be randomly chosen to determine an average IMT for each participant. The nine images will be chosen by randomly selecting three images from three different angles of the CCA. These angles include three images from the anterior view, direct view, and posterior view of the right common carotid artery. A 1 cm IMT segment will be measured to the nearest 1/1000 mm at the point 1 cm proximal to the common carotid bulb using the SonoCalc software. The measurements of the IMT from the near and far walls of the CCA will be combined and averaged for each image. Then the nine separate IMT measurements, consisting of the averaged near and far wall IMT, will be combined and averaged to determine an IMT for each subject.

In certain instances where the carotid segment maybe unclear and the software is unable to identify the intima/lumen surface a manual analysis will be performed using the same process described by Fritz et al. (35). The images of the right common carotid artery will be analyzed at the near and far walls if image clarity permits.

Observations will then be made to draw conclusions for the possible existing relationships between IMT and body fat distribution and body fat percentage. Body fat distribution will be determined by waist circumference measurements and body fat percentage will be determined by DEXA fat mass and fat free mass measurements.

Statistical Analysis

Statistical analysis will be performed using SPSS (Software Package for the Social Sciences) for Windows, Version 10.0. The correlation coefficient will be used to describe the strength of the relationship between the dependent and independent variables. The dependent variable is IMT and the independent variables are body fat percentage as measured by DEXA and fat distribution as measured by WC. For all of the tests, a $p < 0.05$ will be used to determine significance.

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