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Abstract

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BACKGROUND: Microvascular dysfunction (MD: impaired performance of blood flow, tissue perfusion, blood pressure, etc.) is one of the earliest stages in the progression of various chronic diseases. OBJECTIVE: The aim of this study was to determine if a difference in microvascular function existed between two metabolically healthy groups that differed in BMI and waist circumference. DESIGN: This study employed a causal comparative design, with two groups: I) normal weight (n =14, BMI <25 kg/m^2), and II) overweight/obese (n = 12, BMI >28 kg/m^2). METHODS: Microvascular function was assessed by measuring skin blood flow (SkBF) using laser Doppler flowmetry during postocclusive reactive hyperemia (PORH). The area under the SkBF time curve during the 60-second PORH response was used to quantify the magnitude of the microvascular response. RESULTS: Group I (control) had a significantly higher average area under the SkBF time curve (3240 ± 879) than Group II (1948 ± 808) (Z= -3.0094, p = 0.0026). CONCLUSIONS: The overweight/obese subjects exhibited a diminished skin blood flow response to occlusion compared to their normal-weight counterparts. This supports the hypothesis that overweight/obese subjects who are otherwise metabolically healthy exhibit a biological change that is linked to chronic disease.

Keywords: microvascular dysfunction, abdominal obesity, overweight, obese, metabolically healthy, metabolic syndrome

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Introduction

The incidence of overweight/obesity is on the rise and is a major concern in the U.S.[1-3] It has reached epidemic proportions and is a major threat to the overall health and well being of society.[4] According to data from the National Health and Nutrition Examination Survey (NHANES), more than one-third of U.S. adults, over 72 million people, are obese.[2,3] Obesity is related to a number of chronic diseases including hypertension, insulin resistance, type II diabetes mellitus (T2DM), and coronary heart disease (CHD),[5,6] but the underlying mechanisms of obesity's contribution remain unclear. Several studies have found that being overweight also contributes to multiple chronic conditions: gallstones, hypertension, high cholesterol levels, diabetes, and heart disease.[7,8] Exploring the contribution of excess weight to chronic diseases and identifying its mechanisms of action is crucial to developing effective treatments for overweight/obesity-related disorders.

Secondary prevention aims to identify biologic changes in asymptomatic people with risk factors for disease. Overweight/obese people can live for many years without showing symptoms of chronic disease. Identifying biologic changes before symptoms manifest may not prevent the onset of disease, but it could detect diseases at an earlier, more treatable stage. One such biologic change that may exist asymptomatically as a result of obesity is microvascular dysfunction.[9]

Microvascular dysfunction (MD) is known to be present in T2DM, CHD, metabolic syndrome, and obesity;[9,10] however, its presence is not well established in early stages of those diseases. The main functions of microcirculation include the regulation of blood flow and tissue perfusion, blood pressure, tissue fluid (swelling or edema), delivery of oxygen and other nutrients, removal of CO_2 and other metabolic waste products, and body temperature.[11] Under normal conditions, metabolic mechanisms ensure proper regulation of these microcirculatory

functions. Pathologic conditions such as tissue edema, endothelial disturbance, clogging of capillaries by neutrophils and microthrombi, and inflammatory free radicals can compromise these functions and lead to MD.[12,13]

Studies have shown that MD often accompanies excess weight gain.[14-16] Obesity-associated MD is believed to contribute to insulin resistance and hypertension, two leading causes of T2DM and CHD.[9] Some of the symptoms found in obese patients are stunted vasodilation,[17] reduced capillary recruitment,[18] and capillary rarefaction.[19]

One of the most common methods of assessing microvascular function uses laser Doppler flowmetry (LDF), a noninvasive method of measuring microcirculatory blood flow in tissue.[20] Microvascular function is determined by disturbing the normal flow (occlusion or local heat), then measuring the response (typically in the skin), thus testing the vascular system's ability to dilate. Monitoring skin blood flow using laser Doppler flometry (LDF) has been shown to be both valid and reliable as a means for assessing microvascular function.[21]

Thus far, research has been focused primarily on overweight/obesity in general, but as far as we can tell, no studies have compared microvascular function in groups that differ in BMI and waist circumference but lack any other indicator of metabolic syndrome. While obesity is generally believed to be dangerous to overall health, current literature lacks clear evidence of the risks accompanying excess weight gain without associated outward disease states (hypertension, dyslipidemia, T2DM, CHD, etc.). Examining overweight/obese subjects that are otherwise healthy offers a unique view of the underlying dangers excess weight gain can cause. Therefore, the aim of this study was to determine if a difference existed in microvascular function between two presumably metabolically healthy groups that differed in BMI and waist circumference. To fulfill this aim, a group of metabolically healthy and normal-weight adults (nonabdominally

obese; Group I) was compared to a group of overweight/obese adults with abdominal obesity (Group II). The overweight/obese subjects lacked any other indicator of metabolic syndrome. We hypothesized that the microvascular function would be attenuated in the overweight/obese but metabolically healthy group when compared to the normal weight control group.

Methods

Subjects

Volunteers were primarily recruited from the local community and screened, via telephone, to ensure they met the inclusion criteria. We attempted to match age between the two groups by finding a counterpart for each subject who was ±3 years. Originally, 30 subjects were recruited and tested, but four had to be dismissed based on blood test results. Dismissing those subjects led to a slight imbalance in age matching.

Potential subjects possessing any of the following characteristics were excluded from the study: adults over the age of 55 years, smokers, individuals taking vasoactive medications (beta blockers, ACE inhibitors, calcium-channel blockers, diuretics, etc.), postmenopausal women, women on supplemental estrogen or birth control, pregnant or lactating women, individuals with diagnosed heart disease or T2DM, and individuals who exercised vigorously more than three times a week (or >75 total minutes). To estimate physical activity levels, participants were asked to describe their typical exercise habits in an average week. For each listed activity, the participants graded their effort levels based on the Borg Rating of Perceived Exertion Scale. The scale ranges from 6 to 20, where 6 is considered no exertion and 20 is maximal exertion. Anything above a 14 (rated between “somewhat hard” and “hard” on the Borg scale) was considered vigorous.[22]

BYU's Institutional Review Board (IRB) approved this study. Prior to participating in the study, subjects were informed of all potential risks associated with participation and provided written, informed consent. Study participants were placed into one of two groups based on waist circumference and BMI. The control group (Group I) consisted of 14 normal-weight individuals (BMI <25 $kg \cdot m^{-2}$) without any clinical symptoms associated with metabolic syndrome. The comparison group (Group II) was comprised of 12 individuals with abdominal obesity (waist circumference ≥40 inches for men and ≥35 inches for women) and a BMI >28 $kg \cdot m^{-2}$ but no clinical symptoms associated with metabolic syndrome.

Waist circumference and blood pressure were determined in the Human Performance Research Center on Brigham Young University campus. Components of the metabolic syndrome were determined for participants in each group by a lipid profile and fasting glucose test performed at a local hospital laboratory (Mount Timpanogos Hospital, Orem, UT). According to the 2005 International Diabetes Federation definition, metabolic syndrome is present if a patient has abdominal obesity and at least two of the following four conditions: raised triglyceride levels (≥150 $mg \cdot dL^{-1}$), reduced HDL cholesterol (<40 $mg \cdot dL^{-1}$ in males and <50 $mg \cdot dL^{-1}$ in females), raised blood pressure (systolic BP ≥130 or diastolic BP ≥85 $mm \cdot Hg^{-1}$), and raised fasting plasma glucose (≥100 $mg \cdot dL^{-1}$).[23] Two female subjects' HDL levels were 49 $mg \cdot dL^{-1}$, but we chose to include them as this falls within the test's margin of error.

A minimum BMI of 28 $kg \cdot m^{-2}$ was chosen for Group II because the cut points delineating BMI categories (normal, overweight, obese) are somewhat arbitrary as risk lies on a continuum. To increase the likelihood of observing differences between groups, a break in that continuum was necessary (Group I maximum BMI of 24.99 $kg \cdot m^{-2}$ and Group II minimum of 28 $kg \cdot m^{-2}$). Theoretically, the larger the gap between groups the more likely differences can be detected. We

theorized that these cut points would be sufficient to observe a difference in vascular function between groups while allowing for more manageable subject recruitment.

Study Design

Our study employed a causal comparative design. Independent variables were the criteria demarcating each group, including: normal weight and no risk factors (Group I) and overweight/obesity with no other known components of the metabolic syndrome (Group II). The primary dependent variable of interest was the difference in area under the SkBF time curve during the 60-second postocclusive reactive hyperemic (PORH) response; however, the five components of the metabolic syndrome were also compared among groups.

Procedures

In the first appointment, potential subjects completed a participation questionnaire to confirm that they met all the health and physical activity criteria (< 3 days or 75 minutes of vigorous activity per week). Next, we measured blood pressure, waist circumference, height, and weight (to determine BMI). Those subjects who appeared to meet the criteria were sent to a local hospital laboratory to obtain a blood lipid profile and a fasting blood glucose test. Subjects were then assigned to their respective groups based on these test results or excluded from the study. For those who met the criteria, a subsequent appointment was scheduled and completed within a week of laboratory testing.

In the second appointment we assessed microvascular function using laser Doppler flowmetry (LDF), a noninvasive method of measuring microcirculatory blood flow in tissue.[20] Microvascular function is determined by disturbing the normal flow (occlusion or local heat), then measuring the payback response of blood flow, thus testing the vascular system's ability to dilate.

Measurements

We used a standard sphygmomanometer and stethoscope to measure blood pressure. To allow sufficient time for normalization of blood pressure, the first reading was performed after the subject was in a relaxed sitting position for at least five minutes. We ensured the arm was supported at heart level and that the hand was relaxed. Measurements were taken until two consecutive readings differed by no more than 2 $mm \bullet Hg^{-1}$, with a one-minute lapse between readings.[24]

For consistency, we measured waist circumference around the umbilicus, using a flexible, spring-loaded tape measure. Two measurements were taken, but if the difference was greater than 2 cm, a third measurement was taken. Measurements were averaged. To accurately determine subjects' BMI, height (cm) and weight (kg) were measured to the nearest 0.1 cm and 0.01 kg, respectively, using a stadiometer (Seca, Chino, CA) and weight scale (Tanita, Toyko, Japan). Subjects wore only spandex shorts or a swimsuit.

To obtain a lipid profile and a fasting blood glucose test, subjects were sent to a local hospital laboratory. They employ the hexokinase reaction method to determine blood glucose. For the lipid profile, total cholesterol (TC), high-density lipoproteins (HDL), and triglycerides (TGL) are measured. Low-density lipoproteins (LDL) are estimated by the Friedewald formula [Total cholesterol - HDL - (TG/5)], and very-low-density lipoproteins (VLDL) are estimated by subtracting HDL and LDL from the total cholesterol.[25] The lab uses the Dimension Vista System: Flex reagent cartridge by Siemens to measure HDL, TC, and TGL.

All individuals had to abstain from caffeine-containing drinks for 24 hours prior to their second appointment, as it is a vasoactive agent.[26] The cutaneous microvascular response to postocclusion reactive hyperemia (PORH) was assessed as an index of microvascular function.

Skin blood flow (SkBF) was monitored using laser Doppler flowmetry (LDF). Microvascular measurements were conducted after 30 minutes of acclimatization in a quiet, temperature-controlled room (23°C), with the measurements obtained from the dorsal aspect of the nondominant forearm at heart level. SkBF was measured at four skin sites on the upper third of the anterior forearm on skin without dermatological lesions. Each LDF probe was mounted on the skin in a thermostatically controlled heater and held at 34°C during the experiment. After acclimatization to the room temperature we recorded baseline SkBF for 15 minutes. Next, a cuff around the upper arm was inflated to 200 mmHg for 5 minutes. The cuff was rapidly deflated and the PORH response was monitored for 10 minutes.[27,28]

Following PORH, the four skin sites were heated to 44°C for 30 minutes to produce a peak cutaneous vasodilation. We used the peak dilation during local heating to normalize all skin blood flow readings and express the data as a percentage of the peak vasodilation rather than a percentage of baselines.[28]

Data Analysis

PC-SAS (version 9.3, SAS Institute, Inc., Cary, NC) was used to analyze all data. Alpha level was set at $p < 0.05$ for all statistical tests. Descriptive data was summarized with means and standard deviations. Area under the SkBF time curve (AUC) was calculated using the GraphPad Prism 6 software program (San Diego, CA). This program employs the "trapezoidal rule" to find the area under the SkBF time curve, where each small trapezoid within the area is computed and then the total sum of those smaller trapezoids equals the total AUC. The AUC calculation was applied to the 60-second PORH response using normalized SkBF reported as a percentage of peak flow. All four SkBF channels were analyzed and then averaged to yield a single number for each subject. The individual subjects were then averaged for each group and analyzed for

differences between groups. Group differences regarding basic characteristics and metabolic syndrome criteria were determined using the General Linear Model. Area under the SkBF time curve was analyzed for normality within each group using the NPAR1WAY procedure in SAS. This procedure yields several different tests for normality (e.g., Shapiro-Wilk). As the data were not normally distributed, differences in the experimental outcome (area under the SkBF time curve) were analyzed using the nonparametric Wilcoxon-Mann-Whitney test. This test compares independent samples and is the equivalent of a t-test for normally distributed data. Age, a potentially confounding variable, was also shown to be nonnormally distributed using the NPAR1WAY procedure. However, the Wilcoxon-Mann-Whitney test for age did not reveal a difference; thus, was not used as a covariate in the analysis.

Results

Table 1 includes basic group characteristics. Means between the two groups differed significantly in weight ($p = 0.0003$) and BMI ($p < 0.0001$), but not in height or age ($p > 0.65$). It also shows statistics for metabolic syndrome criteria and group values. Mean differences between groups were significant for waist circumference ($p < 0.0001$), but not for any other component of the metabolic syndrome.

The main outcome of interest in this study was the SkBF response to PORH. The area under the SkBF time curve for 60 seconds after the release of the blood pressure cuff averaged 3239 ± 879 SkBF $\%_{peak}$ • sec for Group I, while Group II averaged 1948 ± 808 SkBF $\%_{peak}$ • sec ($Z = -3.0094$, $p = 0.0026$). Age was examined with the Wilcoxon-Mann-Whitney test to find a potential difference between groups, but it was not significant ($p = 0.3790$). A very large effect size of group means for area under the SkBF time curve was also found (Cohen's *d* effect size =

1.53; SE = 0.3). Neither baseline nor peak heat values were significantly different between groups.

Discussion

The objective of this study was to determine if a difference existed in microvascular function between two metabolically healthy groups that differed in BMI and waist circumference. We observed a significant difference in the SkBF response to PORH. In support of our hypothesis, it appears that overweight/obese subjects without compounding risk factors for metabolic syndrome exhibit reduced microvascular function. The reduction in the SkBF response during PORH reflects some degree of MD in this group. In addition, the large effect size suggests a strong ability to detect a difference between groups for the SkBF response to PORH. Seeing a difference in microvascular function between the two groups despite the small sample size is evidence that the effect of excess weight on microvascular function is fairly strong. This offers some unique support that underlying biological changes exist in overweight/ obese subjects despite a lack of outward disease states.

Though our design attempted to control for age by matching, there was a wide range of ages among subjects (18–51). To ensure age did not modify the difference between groups in SkBF response to PORH, we applied the Wilcoxon-Mann-Whitney test to the age data. Since it was not significant, we assume that age did not significantly contribute to the difference we saw in SkBF response.

If we look at the present study in the context of previous studies, it's apparent that overweight/obesity may impair microvascular function in several ways. As noted by de Jongh et al., overweight/obese subjects exhibited stunted vasodilation in response to common endothelium-dependent vasodilators in skin and resistance arteries.[17] Additionally, Arcaro et al.

showed reduced vasodilator function of resistance vessels and capillary recruitment in response to reactive hyperemia and shear stress among obese subjects.[18] As evidenced by the difference in area under the SkBF time curve between the groups in our study, those with elevated BMI's and abdominal obesity also exhibited a similar reduction in vasodilator function in response to reactive hyperemia.

Beyond these functional changes, multiple studies observed structural damages of the microvasculature. Frisbee et al. found that skeletal muscle circulation of obese Zucker rats exhibits decreased capillary density (rarefaction) and structural remodeling.[29,30] Recently, a human study of obese individuals (with and without metabolic syndrome) found a negative correlation of skin capillary density with waist circumference and BMI.[19] As BMI and waist circumference increased, capillary density decreased. While our study did not directly examine structural components of microvasculature, it is possible that rarefaction and structural remodeling may at least partially explain the difference in the PORH response between the two groups. Since there was no significant difference between the two groups in regards to peak heated values, it seems that obesity does not affect all vasodilator mechanisms equally. Reactive hyperemic mechanisms were certainly impaired in the overweight/obese group versus the control group. However, the mechanisms regulating heated dilation were not measurably different between obese and nonobese subjects.

While other studies link obesity with MD,[12,17,18] there is a gap in the research where otherwise metabolically healthy overweight/obese subjects are concerned. Our study was a step in exploring one of many underlying biological changes brought on by excess weight gain. Further exploration of this relationship would prove beneficial to understanding one of the many complications obesity produces.

There is great potential to advance future research in this area. A more varied racial makeup would be more representative of the general population. A third group with advanced stages of chronic disease (T2DM or CHD) could also be added since MD is well documented in these disease states. Such a group would provide another reference point to compare the PORH responses between groups. Overweight/obese subjects who exhibit dysfunction could also be followed over time to monitor any deterioration of vascular function along with progression of chronic diseases. Randomized control trials could also be conducted to evaluate and quantify the effect of various interventions (weight loss, exercise programs, drug therapy, etc.) on vascular function.

Our study had a few limitations. As in correlational studies, a causal-comparative study can identify relationships, but it does not establish causation. Randomized control trials are necessary to establish a temporal cause and effect relationship. The racial makeup of our study (85% Caucasian) decreased the ability to generalize to the population as a whole. During the recruiting stages, participants were asked to describe their typical physical activity habits to ensure they met the criteria. Misrepresentation, intentional or not, is certainly a possibility. Exercise training can improve vascular function, independent of any weight loss,[31] and is thus a potential confounding variable in this study.

Conclusion

A reduction in microvascular function is one of the earliest physiological changes observed prior to overt clinical symptoms of various chronic diseases (hypertension, dyslipidemia, T2DM, CVD, etc.). Determining the conditions that initiate microcirculatory dysfunction would be crucial to understanding the progression of many chronic diseases. Overweight and obesity as a whole is connected to various chronic conditions, MD being one of

them. The subjects in our study with elevated BMI's and waist circumferences exhibited a diminished skin blood flow response to occlusion compared to their normal-weight counterparts. This demonstrates that overweight/obese subjects who are otherwise metabolically healthy exhibit a biological change that is associated with several chronic diseases and appears to be an early biomarker for disease progression. This is an important step in understanding one of many underlying complications caused by excess weight.

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Table 1: Group Characteristics and Criteria Variables Associated with Metabolic Syndrome

Variable	Group I (n = 14)	Group II (n = 12)
Age	27.93 ± 9.98	29.75 ± 10.38
Weight (kg)	65.41 ± 11.13	91.77 ± 14.32*
Height (cm)	172.63 ± 9.25	173.08 ± 10.80
BMI ($kg \cdot m^{-2}$)	21.85 ± 1.99	30.41 ± 1.39*
Waist Circ (cm)	78.15 ± 5.68	102.95 ± 5.25*
SBP ($mm \cdot Hg^{-1}$)	113 ± 7	114 ± 6
DBP ($mm \cdot Hg^{-1}$)	73 ± 7	73 ± 6
Glucose ($mg \cdot dL^{-1}$)	88.3 ± 6.8	88.0 ± 8.9
TC ($mg \cdot dL^{-1}$)	158 ± 18	170 ± 17
LDL ($mg \cdot dL^{-1}$)	92 ± 20	108 ± 20
HDL ($mg \cdot dL^{-1}$)	52 ± 7	47 ± 4
TG ($mg \cdot dL^{-1}$)	96 ± 24	114 ± 17

Values represent mean ± 1 SD
BMI: Body mass index; Circ: circumference; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; Glucose: fasting blood glucose; TC: Total cholesterol; LDL: Low-Density Lipoproteins; HDL: High-Density Lipoproteins; TG: Triglycerides.
*$p < 0.05$ Group II (overweight/obese) different from Group I (control).

Table 2: Microvascular Function Analysis

Variable	Mean (Group I, n = 14)	Mean (Group II, n = 12)	p
Baseline (V)	0.28 ± 0.07	0.24 ± 0.04	0.1127
Peak Heat (V)	1.72 ± 0.53	1.65 ± 0.74	0.6251
AUC (SkBF $\%_{peak}$ • sec)	3239 ± 879	1948 ± 808	0.0026

Values represent mean ± 1 SD
(V): volts; AUC: area under the SkBF time curve analysis