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ABSTRACT

Association Between Expanded Normal Weight Obesity and Insulin Resistance Among U.S. Adults in the National Health and Nutrition Examination Survey

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OBEJCTIVE: The purpose of this investigation was to expand the evaluation of Normal Weight Obesity (NWO) and its association with insulin resistance using a nationally representative sample of U.S. adults.

METHODS: A cross-sectional study including 5,983 subjects was conducted. Body fat percentage was assessed using dual energy X-ray absorptiometry (DXA). Expanded Normal Weight Obesity (eNWO) categories (pairings of BMI and body fat percentage classifications) were determined by standard cut-points for BMI and the gender specific median for body fat percentage. Homeostatic Model Assessment-Insulin Resistance (HOMA-IR) levels were used to index insulin resistance.

RESULTS: Mean ± SE values were as follows: BMI: 27.9 ± 0.2 (women) and 27.8 ± 0.1 (men); body fat percentage: 40.5 ± 0.2 (women) and 27.8 ± 0.2 (men); HOMA-IR: 2.04 ± 0.05 (women) 2.47 ± 0.09 (men). HOMA-IR differed systematically and in a dose-response fashion across all levels of the eNWO categories ($F = 291.3$, $P < 0.0001$). As BMI levels increased, HOMA-IR increased significantly and within each BMI category, higher levels of body fat were associated significantly with higher levels of HOMA-IR.

CONCLUSION: Both high BMI and high body fat percentage are strongly related to insulin resistance. In this study, insulin resistance increased incrementally according to BMI levels primarily and body fat levels secondarily. Consequently, due to the costs associated with precisely measuring body fat, and the accuracy of using BMI independently, we recommend that BMI be used in its standard form to predict insulin resistance and not be supplemented with an estimate of body fat.

Keywords: body fat percentage, HOMA-IR, BMI, metabolic function

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Introduction

Over the past several decades, the number of adults in America with excess body weight has increased substantially.[1] According to body mass index (BMI) data derived from the National Health and Nutrition Examination Survey (NHANES), approximately 44% of adults were overweight or obese in 1976-1980.[2] The latest findings, published in 2014, indicate that the prevalence has increased to 69% of adults,[3] an increase of over 55%. This rising trend is not without serious consequences.

Obesity is a significant risk factor for numerous medical conditions, including insulin resistance and metabolic disease. In a recent paper, Lim et al. showed that obesity, whether measured by BMI or body fat percentage, is highly correlated with insulin resistance, as indexed by elevated homeostatic model assessment (HOMA-IR) levels.[4] Additionally, BMI is a serious risk factor for the development of type 2 diabetes, displaying a dose-response relationship.[5] Research also shows that adults who have normal body weight, but excess body fat, are at increased risk for developing metabolic syndrome and insulin resistance.[6]

Though frequently used to classify obesity, BMI does not measure adiposity. BMI is calculated using only height and weight, not body composition.[7] BMI often misclassifies those with excess adiposity (high body fat percentage) as normal, or healthy.[8] Though BMI has high specificity for predicting high body fat percentage,[9,10] several researchers have found that BMI has low sensitivity for predicting body fat percentage.[9-11]

Many individuals assume that because they have a normal body weight, they are metabolically healthy, and those who are overweight may assume that they are metabolically unhealthy. Though commonly used, body weight, and particularly BMI, is not a high-quality index of health status.

Researchers have tried to remedy the problems associated with using BMI to index overweight and obesity. As a result, the concept of Normal Weight Obesity (NWO) has emerged. NWO, a condition in which individuals are classified as normal weight by BMI, but have excess body fat (defined differently by various researchers), has not been researched extensively, but it seems to be a good predictor of multiple health risks. In recent studies, NWO has been associated with metabolic dysregulation,[12] physical impairment,[13] and cardiovascular mortality.[14]

NWO is a good predictor of insulin resistance. Research by Romero-Corral et al. shows that adults with NWO have four times higher prevalence of metabolic syndrome compared to their counterparts, and that insulin sensitivity tends to decrease as body fat percentage increases.[15] Other researchers have also shown that body fat percentage predicts insulin resistance.[16,17] Research by Madeira et al. indicates that those with NWO have six times greater risk for metabolic syndrome than those without NWO.[6] Additionally, adults with sarcopenic obesity,[18] low muscle mass and elevated body fat, also tend to have higher levels of metabolic syndrome,[19] insulin resistance,[20,21] and several cardiovascular risk factors[20] compared to their counterparts.

Although promising, NWO is a limited index, including just one BMI category (normal weight) and one body fat category (high body fat). There are many other possible BMI and body fat combinations, such as underweight-low body fat (UW-L), underweight-high body fat (UW-H), normal weight-low body fat (NW-L), normal weight-high body fat (NW-H), overweight-low body fat (OW-L), overweight-high body fat (OW-H), obese-low body fat (OB-L), and obese-high body fat (OB-H). In the present study, these categories are referred to as expanded normal weight obesity, eNWO. To date, few if any studies have investigated multiple BMI and body fat category pairings and their relationship with metabolic dysregulation. Moreover, when

researchers have studied some BMI and body fat pairs, they have used bioelectrical impedance[14,19,22] or skinfolds[6] to estimate body fat, as opposed to more precise and reliable measurement tools.

The purpose of this investigation was to expand the evaluation of NWO and its association with insulin resistance using a nationally representative sample of U.S. adults. A secondary purpose was to overcome weaknesses of other obesity and metabolic dysregulation research, particularly to assess body fat percentage using a high-quality measurement method, dual energy x-ray absorptiometry (DXA), and to control for potentially confounding factors, such as age, gender, race, smoking, and physical activity.

Methods

Study Design and Subject Selection

A cross sectional study was performed to examine the relationship between an expanded version of NWO (eNWO) and insulin resistance in U.S. adults. Data on eNWO and insulin resistance were obtained using BMI, body fat percentage, and HOMA-IR from the National Health and Nutrition and Examination Surveys (1999-2006). NHANES is an extensive stratified data set representative of the noninstitutionalized civilian population of the United States. More details, including the datasets and methods, are available at http://www.cdc.gov/nchs/nhanes.htm.

From the data collected by NHANES, subjects ages 20-84 years with information on age, race/ethnicity, gender, BMI, body fat percentage, fasting blood glucose and insulin levels, physical activity, and smoking status were included. The number of subjects who had both insulin and glucose data (used to calculate HOMA-IR) was 12,561. Limiting the data to the age range used in this study, 20-84, resulted in 8,331 subjects. By further narrowing the sample to

nondiabetics and those not taking medication for diabetes, the sample was 7,249. Some subjects had missing data for exposure or potentially confounding variables, or had invalid sample weights, resulting in a total of 5,983 subjects. All measurement procedures below are taken from the published guidelines and procedures used by the National Health and Nutrition Examination Survey.[23-27]

Body Measurements

Weight was taken using a Toledo digital scale while the subject was wearing only underwear, a disposable paper gown, and foam slippers. The participant was instructed to stand still in the center of the scale, hands at side, and looking straight ahead. If the subject weighed more than 440 pounds, two scales were used and the readings from each scale were added together.[23]

Standing height was measured with a fixed stadiometer with a moveable headboard. The subject was required to remove hair ornaments, jewelry, buns, braids, and corn rolls from the top of the head. The subject was instructed to stand on the floor with the heels touching and toes pointing out at about a 60 degree angle while making sure both feet were flat on the floor. The heels, buttocks, shoulder blades, and back of head were to touch the vertical backboard if possible. The head was aligned in the Frankfort horizontal plane, defined as the line from the ear canal to the lower border of the orbit of the eye, parallel to the floor and perpendicular to the backboard. Then the headboard was lowered to the top of the head of the participant with the participant standing as tall as possible while taking a deep breath. The headboard was pressed firmly to compress the hair.[23]

Body Mass Index (BMI)

BMI is a commonly used measure of obesity. BMI is calculated using the following formula: weight (kg) divided by height in meters squared.[24] Underweight is defined as having a BMI <18.5, normal weight is defined as having a BMI between 18.5 and 24.9, overweight includes BMIs from 25.0-29.9, and obesity includes those with a BMI of 30.0 or higher.[24]

Body Composition

Body fat percentage using DXA was a measured variable in the NHANES 1999-2006 surveys only. DXA scans were performed in the mobile examination center. Pregnant females were not scanned. The whole-body DXA scan used a Hologic QDR 4500A fan-beam densitometer (Hologic, Inc., Bedford, MA). Hologic DOS software was used to acquire the scans while scanning in fast mode.[23]

DXA scans provide total body fat and lean muscle mass measurements. Sampled participants taller than 6'5" had to be excluded due to table and room size limitations. If the participant weighed more than 300 pounds, he/she was excluded from the DXA scan due to the weight limitation of the table.[23] Due to these exclusions for large body sizes, missing and invalid DXA data were not missing at random, leading the NHANES analysts to perform multiple imputations to fill in the data for analysis. Details of the multiple imputations are described elsewhere.[28,29]

All metal objects such as jewelry, belts, and snaps were removed from the body (small rings that would not come off were acceptable).[23]

The entire body of the sampled participant had to be within the scan border, including the head. Double-sided Velcro was used to help feet remain in an internally rotated position. The participant reclined flat and straight on the table without a pillow with their arms at their sides,

palms down. If the subject could not lay flat with head slightly supported, he/she was excluded from the exam.[23]

Insulin Resistance

Insulin resistance was indexed using HOMA-IR (fasting insulin (μU/mL) × fasting glucose (mg/dL)/405). Fasting insulin and fasting glucose data were obtained through the NHANES measurements of diabetes profiles.[25] Diabetic individuals (defined as having a fasting blood glucose of ≥126 mg/dL, being told by a physician that one is diabetic, or using insulin or using an oral medication for diabetes) were not included in the study because of their extreme HOMA-IR scores when not taking medication and their regulated values when taking medication.

Subjects assigned to morning sessions were asked to fast for 9 hours. Blood was collected by a certified phlebotomist. Before collection, the phlebotomist administered the fasting questionnaire, which included questions pertaining to the last time the subject ate or drank anything except plain water. Subjects were asked specifically about coffee, tea, alcohol, gum, breath mints, cough drops, antacids, supplements, and other items to ensure proper recall. A blood sample of 89-92 ml was collected. Blood samples were not collected from hemophiliacs, participants who received chemotherapy in the last 4 weeks, or those with rashes, edema, paralysis, wounds, burns, or scar tissue.[25]

Insulin specimens were collected as a serum without anticoagulants or preservatives. Specimens were allowed to clot at room temperature for 15-30 minutes, then centrifuged at 1500 × g for 10 min, then transferred to a 2-mL cryogenic screw-top vial and frozen at -20 degrees C. The specimens were sent in weekly batches in Styrofoam-insulated shipping containers with dry ice to the University of Missouri Diabetes Diagnostic Laboratory via overnight courier. Upon

receipt, the processing laboratory stored the specimens in a -70-degree C freezer until analyzed. No analyses were performed on specimens that thawed. Jouan refrigerated centrifuge models GR4-22 and K110 and a gamma counter were used.[25]

In the 1999-2000 and 2001-2002, insulin radioimmunoassay (RIA), a double-antibody batch method was used to measure insulin levels. The measurements for the 1999-2000 and 2001-2002 data were conducted by the University of Missouri-Columbia.[25]

In the 2003-2004 data collection, the Tosoh AIA-PACK IRI, a two-site immunoenzymometric assay, was used to assess fasting insulin levels.[26] The 2003-2004 measurements were also conducted by the Diabetes Diagnostic Laboratory at the University of Missouri-Columbia.

In the 2005-2006 data collection, the Merocodia Insulin ELISA, a two-site enzyme immunoassay was used.[27] The glucose and insulin measurements in 2005-2006 were conducted by the Fairview Medical Center Laboratory at the University of Minnesota.

Because some of the insulin measurement procedures were changed after the 1999-2002 cycle, in order to allow all of the insulin data to be combined into one data set, a regression equation was created by NHANES. Insulin data from 2003-2004 were used as the reference group.[26]

$$\text{Insulin}_{2003\text{-}2004} = (1.0027 * \text{insulin}_{1999\text{-}2002}) - 2.2934 \ (n = 245, r = 0.981).$$

Similarly, the 2005-2006 insulin data were converted so that it would equal the 2003-2004 data, using the following equation.[27]

$$\text{Insulin}_{2003\text{-}2004} = 1.0526 * \text{Insulin}_{2005\text{-}2006} - 1.5674 \ (n = 189, r = 0.9870)$$

Fasting glucose was measured using a specimen of 1.5 mL plasma with NaF as preservative.[25] Samples of whole blood in measurements of 3 to 5 mL were collected in a

vacuum tube containing glycolytic inhibitors, potassium oxalate, and sodium fluoride and were then immediately centrifuged at 1500 g for 10 min. Plasma was then transferred to a 2-mL cryogenic screw-cap vial and frozen at -70 degrees C. Frozen plasma specimens were shipped weekly in batches to the University of Missouri Diabetes Diagnostic Laboratory via overnight courier.

In 1999-2004, the enzyme hexokinase method was used to catalyze the reaction between glucose and adenosine triphosphatease to form glucose-6-phosphate and adenosine diphosphate, using a rise in NADH concentration as a marker of glucose concentration.[25]

In 2005-2006, glucose concentrations were determined using a hexokinase strategy with a sample blank correction. In order to combine the glucose data, NHANES created a regression equation to convert the 2005-2006 glucose data so that it would be comparable to the data from 1999-2004.[27]

$$Glucose_{1999\text{-}2004} = 0.9835 * Glucose_{2005\text{-}2006},\ n = 92,\ r = 0.9993$$

Expanded Normal Weight Obesity (eNWO)

In order to study the relationship between eNWO and insulin resistance, we formed pairings of BMI and body fat percentage. Underweight-low body fat (UW-L) was defined as having a BMI <18.5 kg/m^2 and a body fat percentage below the overall gender-specific median. Underweight-high body fat (UW-H) was defined as having a BMI <18.5 kg/m^2 and a body fat percentage above the gender-specific median. Normal weight-low body fat (NW-L) was defined as having a normal BMI (18.5-24.9 kg/m^2) and a body fat percentage below the gender-specific median. Normal weight-high body fat (NW-H) was defined as subjects having a normal BMI (18.5-24.9 kg/m^2) and a body fat above the gender-specific median. Overweight-low body fat (OW-L) was defined as having a BMI of 25-29.9 kg/m^2 and a body fat percentage below the

gender-specific median. Overweight-high body fat (OW-H) was defined as having a BMI of 25-29.9 kg/m^2 and a body fat percentage above the gender-specific median. Obese-low body fat (OB-L) was defined as having a BMI ≥30 kg/m^2 and a body fat percentage below the gender-specific median. Obese-high body fat (OB-H) was defined as having a BMI ≥30 kg/m^2 and a high body fat percentage above the gender-specific median.

Covariates

The study controlled for differences in age, gender, race, cigarette smoking, and physical activity. NHANES uses the following race/ethnicity categories: Non-Hispanic White, Non-Hispanic Black, Mexican American, Other Race, including Multi-Racial, and Other Hispanic. Research shows that various demographic factors affect BMI and body composition, including age,[30] gender,[30] and race/ethnicity.[31]

Cigarette smoking was indexed using pack-years. The number of cigarettes smoked per day and the number of years the person has smoked was multiplied and then divided by 20, resulting in a continuous variable, pack years.[32] Research shows that cessation of smoking is associated with weight gain.[33] Research also indicates that smokers weigh less than never-smokers.[34] Additional research shows that smokers tend to be leaner[35] and lighter[36] than nonsmokers. Smokers also tend to have a higher risk of developing insulin resistance and hyperinsulinemia,[37] as well as an increased risk for metabolic syndrome.[38]

NHANES assesses participation in moderate and vigorous physical activity by employing two separate questions.[32] Moderate activity was assessed by asking subjects whether or not they participated in moderate physical activity for at least 10 minutes over the last 30 days. Moderate activity was described as causing only light sweating or a slight to moderate increase in breathing or heart rate, as used in other research.[39,40] Examples included brisk walking, bicycling

for pleasure, golf, and dancing. Involvement in vigorous activity was measured similarly, but vigorous activity was described as causing heavy sweating or large increases in breathing or heart rate.[39] Examples included running, lap swimming, aerobics classes, or fast bicycling. Several investigations indicate that participation in moderate and/or vigorous physical activity are predictive of lower levels of body weight and fat[41,42] and lower risk of insulin resistance.[43]

Data Analysis

Results derived from NHANES research are special and unique because they can be generalized to the U.S. noninstitutionalized civilian population. This is because of the use of sample weights. According to NHANES, a sample weight is assigned to each participant. It is a measure of the number of people in the population represented by that sample person in NHANES, reflecting the unequal probability of selection, nonresponse adjustment, and adjustment to independent population controls. When unequal selection probability is applied, the sample weights can be used to produce an unbiased national estimate.

In the present study, descriptive data, including frequencies for categorical variables and means ± standard errors for continuous variables, were reported. Each descriptive value included adjustments based on the complex sampling design of NHANES by incorporating strata and primary sampling unit (PSU) indicators, as well as sample weights for the subsample of fasting participants used in the current study. Proc SurveyMeans was employed to generate weighted means that represent values for the U.S. population, and Proc SurveyFreq was used to calculate weighted frequencies, which are also generalizable to the U.S. adult population.

The primary outcome variable of the current study was insulin resistance, indexed using HOMA-IR. Individuals with fasting blood glucose levels > 126 mg/dL, signifying diabetes, and participants who reported being told by their physician that they had diabetes, were removed

from the data set because medications affecting insulin and glucose would significantly affect HOMA-IR levels. Similarly, individuals reporting that they take insulin or other medications to control their blood sugar levels were not included in the analyses.

Because HOMA-IR distributions deviated significantly from normality, HOMA-IR values were transformed by natural logarithm prior to modeling. To aid interpretation of the results, untransformed HOMA-IR values were reported in this study.

For the current study, the exposure variable was a version of normal weight obesity expanded to include all categories of BMI (eNWO), which has not been investigated in past studies. The typical BMI categories (underweight, normal weight, overweight, and obese) were used along with two categories based on body fat percentage (low body fat and high body fat). The eNWO was a categorical variable reflecting each possible pairing between the four BMI categories and the two categories of body fat. In total, there were eight eNWO categories, as follows: underweight-low body fat, underweight-high body fat, normal weight-low body fat, normal weight-high body fat, overweight-low body fat, overweight-high body fat, obese-low body fat, and obese-high body fat. Both underweight categories were combined into one general underweight category because the number of subjects in each was extremely low.

The extent to which mean HOMA-IR values differed across the categories of eNWO was determined using linear regression analysis and the Proc SurveyReg procedure. Estimates from each regression model were based on the complex, multistage, probability sampling process of NHANES and incorporated the strata, primary sampling units, and sample weights for the fasting subsample used in the present study. To test the hypothesis that the association between eNWO and insulin resistance is partially mediated by differences in age, gender, and race, these factors were controlled statistically using partial correlation. The potential confounding effects of

cigarette smoking, moderate physical activity, and vigorous physical activity were also tested using partial correlation and the Proc SurveyReg procedure. Adjusted means were calculated using the least-squares means procedure.

All P-values were two-sided and statistical significance was accepted when alpha was < 0.05. The statistical analyses were computed using SAS Version 9.4 (SAS Institute, Inc., Cary, NC).

Results

Descriptive information about the sample of 5,983 participants is displayed in Table 1. In brief, more participants fit the normal weight-low body fat category than any other, although prevalence within the obese-high body fat group was similar. Overall, nearly two-thirds of the sample was overweight or obese, according to typical BMI cut-points. Additionally, 55% of the sample reported that they did not get at least 10 minutes of moderate-intensity physical activity in the past 30 days and over 60% reported that they did not get 10 minutes of vigorous-intensity activity in the past 30 days.

Mean BMI (± SE) was 27.9 ± 0.2 kg/m^2 for women and 27.8 ± 0.1 kg/m^2 for men, whereas average body fat percentage was 40.5 ± 0.2 and 27.8 ± 0.2 for women and men, respectively. Mean HOMA-IR was 2.04 ± 0.05 for women and 2.47 ± 0.09 for men.

As shown in Table 2, with the BMI and body fat measures combined, HOMA-IR levels differed systematically and in a dose-response fashion across all seven of the eNWO categories ($F = 291.3$, $P < 0.0001$). As BMI levels increased, HOMA-IR increased significantly, and within each BMI category, higher levels of body fat were associated significantly with higher levels of HOMA-IR. Adjusting for differences in the demographic covariates, specifically age, gender, race, and year of assessment, adjusted the relationship between eNWO and HOMA-IR slightly

(F = 286.2, P < 0.0001). However, controlling for differences in the lifestyle covariates, in addition to the demographic variables, strengthened the association between eNWO and HOMA-IR (F = 340.9, P < 0.0001).

HOMA-IR differences between the high- and low-body-fat categories within each BMI level were meaningful. For example, within the normal weight BMI category, the HOMA-IR mean for the high body fat group was approximately 45% higher than it was for those in the low body fat category.

As shown in Table 3, with subjects divided into BMI categories based on sex-specific quintiles, HOMA-IR differed significantly across the five BMI groups (BMI5) with no variables controlled (F = 448.5, P < 0.0001). With participants divided into body-fat-percentage categories based on sex-specific quintiles (BF%5) and no variables controlled, the relationship with HOMA-IR was similar (F = 451.6, P < 0.0001), as displayed in Table 4. Adjusting for differences in all of the demographic and lifestyle covariates resulted in stronger associations between HOMA-IR and BMI5 (F = 464.4, P < 0.0001) and BF%5 (F = 511.3, P < 0.0001), as shown in Table 3 and Table 4, respectively. The relationship between BMI5 and HOMA-IR was weakened substantially when all of the covariates and also BF%5 were controlled statistically (F = 141.9, P < 0.0001), as shown in Table 3. However, the association between BF%5 and HOMA-IR was weakened to a greater extent after adjusting for all of the covariates and also BMI5 (F = 68.8, P < 0.0001), as shown in Table 4.

The relationship between BMI and body fat percentage was moderate (R^2 = 0.325, F = 2194.4, P < 0.0001) with both variables treated as continuous variables. With BMI and body fat percentage divided into sex-specific quintiles, the association was also significant (Wald Chi-Square = 1411.0, F = 88.2, P < 0.0001). Agreement among quintiles was modest. There was 67%

agreement between quintile 1 for BMI5 and quintile 1 for BF%5. For quintile 5, agreement was similar (66%) for BMI5 and BF%5. However, for quintile 2, agreement was 37% and for quintile 3, agreement was 33%. For quintile 4, agreement was 38%.

Discussion

Obesity is a major risk factor for numerous medical conditions, including insulin resistance.[4] Further, an elevated BMI significantly increases risk for developing type 2 diabetes.[5] Some investigations have shown that adults with normal BMIs but excess body fat, normal weight obesity (NWO), are also at increased risk for developing metabolic syndrome and insulin resistance.[6] The purpose of the present study was to expand the concept of NWO (eNWO) to include all BMI categories, along with a precise measure of body fat, and investigate the relationship between eNWO and insulin resistance.

According to the results, there appears to be an undeviating dose-response relationship for insulin resistance across each of the seven eNWO categories. Specifically, with each BMI category divided into low- and high-body-fat groups, HOMA-IR varies according to body fat levels within each BMI category. This pattern remains consistent across the entire eNWO spectrum, without exception, and each HOMA-IR mean differs significantly from each other mean across every eNWO category (Table 2).

If body fat plays a more important role than BMI in insulin resistance, as suggested by Gomez-Ambrosi,[44] one would expect individuals with low body fat to have lower HOMA-IR levels than those in a neighboring lower BMI category with high body fat. For example, if body fat was key, then logic would suggest that participants in the overweight-low body fat category would tend to have lower HOMA-IR levels than those in the normal weight-high body fat category. However, this was not supported by the present study. Instead, HOMA-IR moved

incrementally according to BMI levels primarily and body fat levels secondarily. Specifically, individuals in the overweight-low body fat category had significantly higher HOMA-IR levels than those in the normal weight-high body fat category, and this pattern persisted across all of the eNWO categories.

Testing for differences in HOMA-IR across the gender-specific BMI (Table 3) and body-fat-percentage (Table 4) quintiles separately showed that the two body composition indexes have similar independent associations with HOMA-IR. However, after adjusting for differences in the covariates and BMI5 (BMI quintile), the relationship between body fat percentage quintile (BF%5) and HOMA-IR was attenuated more than when the covariates and BF%5 were controlled and the association between BMI5 and HOMA-IR was tested. Apparently, the relationship between BMI and HOMA-IR is much stronger after adjusting for body fat differences than the association between body fat and HOMA-IR with BMI controlled. Specifically, with no variables controlled statistically, mean HOMA-IR for the fifth quintile of BMI was approximately 4x greater than the mean for the first BMI quintile. However, after adjusting for all of the covariates and BF%5, mean HOMA-IR for the fifth BMI quintile was 2.7x greater than the first BMI quintile. On the other hand, with no variables controlled, mean HOMA-IR for the fifth quintile of BF% was approximately 4x greater than the first quintile of BF%. However, after adjusting for all of the covariates and BMI5, mean HOMA-IR for the fifth BF% quintile was only 1.6x greater than the HOMA-IR mean of the first BF% quintile.

Although both BMI and body fat percentage appear to play important roles in insulin resistance, NHANES data suggests that the contribution of BMI is greater than the contribution of body fat percentage. Therefore, given the costs of time, training, and equipment associated with measuring body fat to supplement BMI, and also the fact that BMI results remain the same

whether or not body fat findings are included, it seems that for predicting HOMA-IR, the better choice is to differentiate adults according to BMI and not include a measure of body fat percentage.

To date, a limited number of studies have investigated the relationship between NWO and insulin resistance. However, few have used a reliable measure of body fat, such as DXA scans, and none have examined the spectrum of categories included in eNWO. The studies which investigated only the NWO category compared to a non-NWO category had similar results to the present study in that individuals with normal weight and high body fat had higher rates of metabolic dysfunction than those with normal weight and low body fat. For example, Madeira et al. investigated NWO and its relationship with insulin resistance and found that the presence of NWO, measured by skinfolds, was correlated with low insulin sensitivity compared to those of a normal weight without high body fat.[6] Additionally, Romero-Corral et al. studied the relationship between NWO and metabolic syndrome, and found that NWO, when measured by bioelectrical impedance, was associated with four times the prevalence of metabolic syndrome compared to those in a normal weight-low body fat group.[15] Batsis found that NWO, indexed using bioelectrical impedance, predicted prevalence of higher insulin resistance when using tertiles, but not cut-points, for body fat.[14] In another investigation which used air displacement plethysmography to determine body fat percentage, findings showed that NWO predicted higher HOMA-IR levels.[16] Lastly, only one other study has used DXA to measure body fat, and this investigation found that NWO was associated with higher insulin resistance, but, like the others, it did not look at categories beyond normal weight-high body fat and normal weight-low body fat.[17]

It appears that the present study is consistent with the findings of previous research conducted to compare insulin resistance among adults with normal weight and high body fat to those with normal weight and low body fat. However, no other investigations have evaluated normal weight obesity in its expanded form.

Potential Mechanisms

Currently, there is little known concerning the mechanisms associated with NWO as it relates to insulin resistance.[45] However, there are a few potential mechanisms. First, in general, it appears that women with NWO generally tend to have higher levels of cardiovascular risk factors, including blood pressure, lipids, and hyperglycemia compared to lean women.[45] Additionally, plasma leptin concentrations are correlated with BMI and body fat levels.[45] Research shows that women with NWO have higher leptin levels than lean women, but lower levels than women with obesity.[45] Additionally, it is possible that absolute amount of fat mass, rather than body fat percentage, plays a role in the relationship with HOMA-IR.

Another possible mechanism, as discussed by Jean et al., is that metabolically obese normal-weight individuals may tend to be more sedentary or have increased carbohydrate intake compared to their counterparts, which could lead to increased fat cell size and insulin secretion.[46] Further, the amount of adipose tissue in the body significantly affects hormone levels in the body[47] and various hormones released by the adipose tissue play an important role in metabolic complications.[48] Additionally, adipose cell size is predictive of metabolic dysregulation.[49,50]

Energy intake may be a moderating factor,[51] affecting the relationship between insulin resistance and eNWO. Insulin resistance denotes a physiologic adaptation that may restrict the further storing of fat.[51] Some studies indicate that insulin resistance may protect against weight

gain when body weight levels are extreme, but other studies show conflicting results.[51]

Limitations and Strengths

The present study had some limitations. Due to the cross-sectional nature of the study, causality or directionality of the relationship between insulin resistance, BMI, and body fat percentage could not be determined. It is possible that a high BMI or high body fat percentage may cause an increase in insulin resistance, or insulin resistance may lead to a change in BMI or body fat percentage. Moreover, the present study controlled for several potential confounding variables, including age, gender, race, physical activity, and smoking, but there is always a possibility that an unknown lurking variable, not controlled in this investigation, was responsible for the relationship between eNWO and insulin resistance. Accuracy of self-reported variables was another potential limitation. Assessment of physical activity and smoking habits were both self-reported, and therefore may contain error due to misreporting.

A strength of the present study was its large sample size representing noninstitutionalized civilians in the United States. As the sample represents virtually all of the United States adult population, the results are much more generalizable than previous studies investigating NWO. Another strength was the use of DXA, a reliable and precise measure of body fat. Previous research has employed skinfolds, bioelectrical impedance, and other methods lacking the precision and reliability of DXA. Lastly, the present study expanded NWO, allowing the effect of body fat to be studied across each level of BMI. The concept of NWO has never been expanded before.

In conclusion, both high BMI and high body fat percentage are strongly related to insulin resistance. However, according to the present study based on NHANES data, insulin resistance increases incrementally according to BMI levels primarily and body fat levels secondarily.

Consequently, due to the costs associated with precisely measuring body fat and the accuracy of using BMI independently, we recommend that BMI be used in its standard form to predict insulin resistance and not be supplemented with an estimate of body fat.

References

1. Poirier P, Giles TD, Bray GA, et al. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss. *Arterioscler Thromb Vasc Biol.* 2006;26(5):968-976.

2. Flegal KM, Carroll MD, Kuczmarski RJ, et al. Overweight and obesity in the United States: prevalence and trends, 1960-1994. *Int J Obes Relat Metab Disord* 1998;22(1):39-47.

3. Ogden CL, Carroll MD, Kit BK, et al. Prevalence of childhood and adult obesity in the United States, 2011-2012. *JAMA* 2014;311(8):806-814.

4. Lim SM, Choi DP, Rhee Y, et al. Association between Obesity Indices and Insulin Resistance among Healthy Korean Adolescents: The JS High School Study. *PLoS One* 2015;10(5):e0125238.

5. Hillier TA, Pedula KL. Characteristics of an adult population with newly diagnosed type 2 diabetes: the relation of obesity and age of onset. *Diabetes Care* 2001;24(9):1522-1527.

6. Madeira FB, Silva AA, Veloso HF, et al. Normal weight obesity is associated with metabolic syndrome and insulin resistance in young adults from a middle-income country. *PLoS One* 2013;8(3):e60673.

7. De Lorenzo A, Bianchi A, Maroni P, et al. Adiposity rather than BMI determines metabolic risk. *Int J Cardiol* 2013;166(1):111-117.

8. Gallagher D, Heymsfield SB, Heo M, et al. Healthy percentage body fat ranges: an approach for developing guidelines based on body mass index. *Am J Clin Nutr* 2000;72(3):694-701.

9. Okorodudu DO, Jumean MF, Montori VM, et al. Diagnostic performance of body mass index to identify obesity as defined by body adiposity: a systematic review and meta-analysis. *Int J Obes* 2010;34(5):791-799.

10. Romero-Corral A, Somers VK, Sierra-Johnson J, et al. Accuracy of body mass index in diagnosing obesity in the adult general population. *Int J Obes* 2008;32(6):959-966.

11. Baumgartner RN, Heymsfield SB, Roche AF. Human body composition and the epidemiology of chronic disease. *Obes Res* 1995;3(1):73-95.

12. Oliveros E, Somers VK, Sochor O, et al. The concept of normal weight obesity. *Prog Cardiovasc Dis* 2014;56(4):426-433.

13. Batsis JA, Sahakyan KR, Rodriguez-Escudero JP, et al. Normal weight obesity and functional outcomes in older adults. *Eur J Intern Med* 2014;25(6):517-522.

14. Batsis JA, Sahakyan KR, Rodriguez-Escudero JP, et al. Normal weight obesity and mortality in United States subjects >/=60 years of age (from the Third National Health and Nutrition Examination Survey). *Am J Cardiol* 2013;112(10):1592-1598.

15. Romero-Corral A, Somers VK, Sierra-Johnson J, et al. Normal weight obesity: a risk factor for cardiometabolic dysregulation and cardiovascular mortality. *Eur Heart J* 2010;31(6):737-746.

16. Gomez-Ambrosi J, Silva C, Galofre JC, et al. Body mass index classification misses subjects with increased cardiometabolic risk factors related to elevated adiposity. *Int J Obes* 2012;36(2):286-294.

17. Kim MK, Han K, Kwon H-S, et al. Normal weight obesity in Korean adults. *Clin Endocrinol* 2014;80(2):214-220.

18. Cauley JA. An Overview of Sarcopenic Obesity. *J Clin Densitom* 2015;18(4):499-505.

19. Lu CW, Yang KC, Chang HH, et al. Sarcopenic obesity is closely associated with metabolic syndrome. *Obes Res Clin Pract* 2013;7(4):e301-307.

20. Chung JY, Kang HT, Lee DC, et al. Body composition and its association with cardiometabolic risk factors in the elderly: a focus on sarcopenic obesity. *Arch Gerontol Geriatr* 2013;56(1):270-278.

21. Kim TN, Park MS, Lim KI, et al. Relationships between sarcopenic obesity and insulin resistance, inflammation, and vitamin D status: the Korean Sarcopenic Obesity Study. *Clin Endocrinol* 2013;78(4):525-532.

22. Romero-Corral A, Somers VK, Sierra-Johnson J, et al. Normal weight obesity: a risk factor for cardiometabolic dysregulation and cardiovascular mortality. *Eur Heart J* 2010;31(6):737-746.

23. National Health and Nutrition Examination Survey Antrhopometry Procedures Manual. Centers for Disease Control and Prevention Web site. Available from: http://www.cdc.gov/nchs/data/nhanes/bm.pdf

24. ACSM. ACSM's Guidelines for Exercise Testing and Prescription. 9th ed. Baltimore, MD: American College of Sports Medicine 2014.

25. Laboratory Procedure Manual. Centers for Disease Control and Prevention Web site. Available from: http://www.cdc.gov/nchs/data/nhanes/nhanes_01_02/l10am_b_met_insulin.pdf

26. 2003-2004 Data Documentation, Codebook, and Frequencies. Centers for Disease Control and Prevention Web site. Available from: http://wwwn.cdc.gov/Nchs/Nhanes/2003-2004/L10AM_C.htm

27. 2005-2006 Data Documentation, Codebook, and Frequencies. Centers for Disease Control and Prevention Web site. Available from: http://wwwn.cdc.gov/Nchs/Nhanes/2005-2006/GLU_D.htm

28. Schenker N, Borrud LG, Burt VL, et al. Multiple imputation of missing dual-energy X-ray absorptiometry data in the National Health and Nutrition Examination Survey. *Stat Med* 2011;30(3):260-276.

29. The 1999-2006 Dual Energy X-ray Absorptiometry (DXA) Multiple Imputation Data Files and Technical Documentation. Centers for Disease Control and Prevention Web site. Available from: http://wwwn.cdc.gov/Nchs/Nhanes/Dxx/dxa.aspx

30. Jackson AS, Stanforth PR, Gagnon J, et al. The effect of sex, age and race on estimating percentage body fat from body mass index: The Heritage Family Study. *Int J Obes Relat Metab Disord* 2002;26(6):789-796.

31. Deurenberg P, Yap M, van Staveren WA. Body mass index and percent body fat: a meta analysis among different ethnic groups. *Int J Obes Relat Metab Disord* 1998;22(12):1164-1171.

32. NHANES 1999-2000 Questionnaire Data. Centers for Disease Control and Prevention Web site. Available from: http://wwwn.cdc.gov/Nchs/Nhanes/Search/DataPage.aspx?Component=Questionnaire&CycleBeginYear=1999

33. Kawachi I, Troisi RJ, Rotnitzky AG, et al. Can physical activity minimize weight gain in women after smoking cessation? *Am J Public Health* 1996;86(7):999-1004.

34. Koster-Rasmussen R, Permin CA, Siersma V, et al. Back on track-Smoking cessation and weight changes over 9years in a community-based cohort study. *Prev Med* 2015;81:320-325.

35. Klesges RC, Stein RJ, Hultquist CM, et al. Relationships among smoking status, body composition, energy intake, and physical activity in adult males: a longitudinal analysis. *J Subst Abuse* 1992;4(1):47-56.

36. Moffatt RJ, Owens SG. Cessation from cigarette smoking: changes in body weight, body composition, resting metabolism, and energy consumption. *Metabolism* 1991;40(5):465-470.

37. Haj Mouhamed D, Ezzaher A, Neffati F, et al. Effect of cigarette smoking on insulin resistance risk. *Ann Cardiol Angeiol (Paris)* 2015.

38. Cibickova L, Karasek D, Langova K, et al. Correlation of lipid parameters and markers of insulin resistance: does smoking make a difference? *Physiol Res* 2014;63 Suppl 3:S387-393.

39. Needham BL, Adler N, Gregorich S, et al. Socioeconomic status, health behavior, and leukocyte telomere length in the National Health and Nutrition Examination Survey, 1999-2002. *Soc Sci Med* 2013;85:1-8.

40. Wei Y, Zhu J, Nguyen A. Urinary concentrations of dichlorophenol pesticides and obesity among adult participants in the U.S. National Health and Nutrition Examination Survey (NHANES) 2005-2008. *Int J Hyg Environ Health* 2014;217(2-3):294-299.

41. Donnelly JE, Hill JO, Jacobsen DJ, et al. Effects of a 16-month randomized controlled exercise trial on body weight and composition in young, overweight men and women: the Midwest Exercise Trial. *Arch Intern Med* 2003;163(11):1343-1350.

42. Irving BA, Davis CK, Brock DW, et al. Effect of exercise training intensity on abdominal visceral fat and body composition. *Med Sci Sports Exerc* 2008;40(11):1863-1872.

43. Khoo J, Dhamodaran S, Chen DD, et al. Exercise-induced weight loss is more effective than dieting for improving adipokine profile, insulin resistance and inflammation in obese men. *Int J Sport Nutr Exerc Metab* 2015; 25(6):566-75.

44. Gomez-Ambrosi J, Silva C, Galofre JC, et al. Body adiposity and type 2 diabetes: increased risk with a high body fat percentage even having a normal BMI. *Obesity (Silver Spring)*. 2011;19(7):1439-1444.

45. Marques-Vidal P, Pecoud A, Hayoz D, et al. Normal weight obesity: relationship with lipids, glycaemic status, liver enzymes and inflammation. *Nutr Metab Cardiovasc Dis* 2010;20(9):669-675.

46. Jean N, Somers V, Sochor O, et al. Normal-Weight Obesity: Implications for Cardiovascular Health. *Curr Atheroscler Rep* 2014;16(12):1-8.

47. Nway NC, Sitticharoon C, Chatree S, et al. Correlations between the expression of the insulin sensitizing hormones, adiponectin, visfatin, and omentin, and the appetite regulatory hormone, neuropeptide Y and its receptors in subcutaneous and visceral adipose tissues. *Obes Res Clin Pract* 2015.

48. You T, Wang X, Murphy KM, et al. Regional Differences in Adipose Tissue Hormone/Cytokine Production Before and After Weight Loss in Abdominally Obese Women. *Obesity (Silver Spring)* 2014;22(7):1679-1684.

49. McLaughlin T, Craig C, Liu LF, et al. Adipose Cell Size and Regional Fat Deposition as Predictors of Metabolic Response to Overfeeding in Insulin-Resistant and Insulin-Sensitive Humans. *Diabetes* 2016.

50. Eriksson-Hogling D, Andersson DP, Backdahl J, et al. Adipose tissue morphology predicts improved insulin sensitivity following moderate or pronounced weight loss. *Int J Obes (Lond)* 2015;39(6):893-898.

51. Tucker LA, Tucker JM. Insulin resistance as a predictor of gains in body fat, weight, and abdominal fat in nondiabetic women: a prospective study. *Obesity* 2012;20(7):1503-1510.

Table 1 Descriptive characteristics of the sample and mean HOMA-IR for each category (n = 5,983)

Categorical Variable	N	Weighted %	Mean HOMA	SE	F	P
eNWO					291.3	<0.0001
Underweight	94	1.9	0.8[a]	0.07		
Normal weight-low body fat	1624	30.1	1.1[b]	0.02		
Normal weight-high body fat	288	4.5	1.6[c]	0.08		
Overweight-low body fat	927	15.9	1.9[d]	0.05		
Overweight-high body fat	1221	18.3	2.2[e]	0.07		
Obese-low body fat	141	2.1	3.2[f]	0.28		
Obese-high body fat	1688	27.2	3.9[g]	0.14		
Gender					34.2	<0.0001
Men	2939	50.8	2.5	0.09		
Women	3044	49.2	2.0	0.05		
Race					15.0	<0.0001
Non-Hispanic White	2468	55.8	2.2[a]	0.09		
Non-Hispanic Black	847	7.9	2.5[b,d]	0.07		
Mexican American	1072	5.5	2.6[c,d]	0.08		
Other Race	1401	26.8	2.3[a]	0.08		
Other Hispanic	195	3.9	2.4[d]	0.23		
Year of Assessment					4.0	0.0114
1999-2000	1489	23.1	2.2[a,b]	0.08		
2001-2002	1679	26.9	2.1[b,c]	0.06		
2003-2004	1540	26.0	2.5[a]	0.16		
2005-2006	1275	23.9	2.3[c]	0.09		
Moderate Physical Activity					14.2	0.0004
Yes	3055	45.0	2.2	0.07		
No	2928	55.0	2.4	0.06		
Vigorous Physical Activity					30.12	<0.0001
Yes	2000	38.2	2.0	0.06		
No	3983	61.8	2.4	0.07		

eNWO = expanded normal weight obesity category

Low body fat is defined as below the overall gender-specific median, and high body fat is defined as above the overall gender-specific median.

In the column titled Weighted %, summing the values may not equal 100% due to rounding.

The F values reflect mean HOMA-IR differences across each categorical variable without adjusting for any covariates.

For each variable, means with the same superscript letter in the Mean HOMA column indicate that the means are not significantly different (P > 0.05).

Table 2 Mean differences in HOMA-IR across the expanded normal weight obesity (eNWO) categories, adjusted for covariates.

	Expanded Normal Weight Obesity (eNWO)								
	UW	NW-L	NW-H	OW-L	OW-H	OB-L	OB-H		
Covariates	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	F	P
None	0.8 ± 0.07[a]	1.1 ± 0.02[b]	1.6 ± 0.08[c]	1.9 ± 0.05[d]	2.2 ± 0.07[e]	3.2 ± 0.28[f]	3.9 ± 0.14[g]	291.3	<0.0001
Demographics*	0.9 ± 0.09[a]	1.2 ± 0.05[b]	1.7 ± 0.08[c]	1.8 ± 0.07[d]	2.3 ± 0.08[e]	3.2 ± 0.28[f]	4.0 ± 0.13[g]	286.2	<0.0001
Demographics and Lifestyle[†]	0.9 ± 0.09[a]	1.1 ± 0.05[b]	1.6 ± 0.09[c]	1.8 ± 0.07[d]	2.2 ± 0.09[e]	3.2 ± 029[f]	3.9 ± 0.12[g]	340.9	<0.0001

UW = underweight
NW-L = normal weight-low body fat
NW-H = normal weight-high body fat
OW-L = overweight-low body fat
OW-H = overweight-high body fat
OB-L = obese-low body fat
OB-H = obese-high body fat
Each mean on the same row is significantly different from each other mean ($P < 0.05$).
*Demographic covariates included: age, gender, race, and year of assessment.
[†]Lifestyle covariates included: moderate physical activity, vigorous physical activity, and smoking.
Means on the same row have been adjusted for differences in the covariates listed in the first column.
Means ± SE on the same row with different superscript letters are significantly different from each other.

Table 3 Mean HOMA-IR values across BMI quintiles without and with control of potential confounders

Variable controlled	BMI Quintiles[§] Quintile 1 Mean ± SE	Quintile 2 Mean ± SE	Quintile 3 Mean ± SE	Quintile 4 Mean ± SE	Quintile 5 Mean ± SE	F	P
None	1.04 ± 0.03[a]	1.36 ± 0.03[b]	1.94 ± 0.05[c]	2.60 ± 0.08[d]	4.31 ± 0.17[e]	448.54	<0.0001
Demographics*	1.10 ± 0.06[a]	1.41 ± 0.05[b]	1.99 ± 0.06[c]	2.65 ± 0.10[d]	4.37 ± 0.16[e]	431.10	<0.0001
Demographics and Lifestyle[†]	1.11 ± 0.05[a]	1.42 ± 0.05[b]	2.01 ± 0.06[c]	2.65 ± 0.10[d]	4.35 ± 0.16[e]	464.39	<0.0001
Demographics, Lifestyle, BF%5	1.48 ± 0.06[a]	1.59 ± 0.05[b]	2.02 ± 0.06[c]	2.50 ± 0.10[d]	3.95 ± 0.14[e]	141.88	<0.0001

[§]Gender specific quintile
*Demographic covariates included: age, gender, race, and year of assessment.
[†]Lifestyle covariates included: moderate physical activity, vigorous physical activity, and smoking.
Means on the same row have been adjusted for differences in the covariates listed in the first column.
Each mean on the same row is significantly different from each other mean ($P < 0.05$).
BF%5 represents the variable, body fat percentage, divided into quintiles.
Means ± SE on the same row with different superscript letters are significantly different from each other.

Table 4 Mean HOMA-IR values across body fat percentage quintiles without and with control of potential confounders

Variable controlled	Body Fat Percentage Quintiles[§] Quintile 1 Mean ± SE	Quintile 2 Mean ± SE	Quintile 3 Mean ± SE	Quintile 4 Mean ± SE	Quintile 5 Mean ± SE	F	P
None	0.99 ± 0.03[a]	1.55 ± 0.5[b]	2.16 ± 0.07[c]	2.60 ± 0.07[d]	3.92 ± 0.17[e]	451.61	<0.0001
Demographics*	0.98 ± 0.45[a]	1.59 ± 0.07[b]	2.24 ± 0.08[c]	2.72 ± 0.08[d]	4.06 ± 0.15[e]	448.21	<0.0001
Demographics and Lifestyle[†]	1.01 ± 0.05[a]	1.60 ± 0.07[b]	2.25 ± 0.08[c]	2.71 ± 0.08[d]	4.04 ± 0.15[e]	511.33	<0.0001
Demographics, Lifestyle, BMI5	1.80 ± 0.07[a]	2.10 ± 0.08[b]	2.38 ± 0.08[c]	2.34 ± 0.07[c]	2.91 ± 0.11[d]	68.83	<0.0001

[§]Gender specific quintile
*Demographic covariates included: age, gender, race, and year of assessment.
[†]Lifestyle covariates included: moderate physical activity, vigorous physical activity, and smoking.
Means on the same row have been adjusted for differences in the covariates listed in the first column.
Each mean on the same row is significantly different from each other mean ($P < 0.05$).
BMI5 represents the variable, body mass index, divided into quintiles.
Mean ± SE on the same row with different superscript letters are significantly different from each other.