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Serum Adiponectin and Ghrelin, Metabolic Syndrome and Diabetes Status in Cuban Americans

Abstract

Purpose: Metabolic syndrome (MetS) is associated with the development of cardiovascular disease (CVD) and type 2 diabetes. Decreases in circulating adiponectin and ghrelin have been associated with MetS. Our primary aim was to evaluate the relationship of MetS with adiponectin and ghrelin for Cuban Americans with and without type 2 diabetes.

Methods: Cross-sectional study of 367 adults, self identified as Cuban extraction and randomly recruited from a mailing list of Broward and Miami-Dade counties. Fasted whole blood for adiponectin (ADPN) was collected using K_3EDTA tubes and measured by ELISA. Ghrelin was assayed with fasted blood plasma by Enzyme Immunometric Assay. MetS and 10-year risk for coronary heart disease (CHD) were determined using the ATP III criteria.

Results: Adiponectin ($F=51.8$, $R^2=0.21$ $p<0.001$) and ghrelin ($F=12.77$, $R^2=0.06$, $p<0.001$) differed by diabetes status (ANOVA) not age and gender. In stepwise linear regression models triglyceride levels $\geq$ 150 mg/dL negatively corresponded (coefficient = -0.23) with ghrelin levels for persons without diabetes ($F=7.45$, $R^2=0.053$, $p=0.007$); abdominal obesity and fasting plasma glucose predicted high sensitivity C-reactive protein (hs-CRP) for persons with and without diabetes ($F=16.3$, $R^2 = 0.144$, $p <0.001$).

Conclusion: Low ghrelin levels were associated with MetS regardless of diabetes status. High adiponectin levels were related to a low probability for those without diabetes only. There was a positive association of hs-CRP with BMI, MetS and number of MetS components.

Keywords: Metabolic syndrome, Type 2 diabetes, Cuban, Adiponectin, Ghrelin, High-sensitivity C-reactive protein

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Introduction

It is estimated that 45.5 million Hispanics reside in the United States, making people of Hispanic origin the nation's largest ethnic or race minority (14.7%) [1]. The percentage of Hispanic-origin people in households who are Cuban is 3.5% [1]. More than half (68.5%) of the nation's Cubans live in Miami-Dade County, Florida [1]. Elderly [equal or greater than 65 years of age] Cuban Americans represent the largest segment (21%) of the elderly population among the US ethnic/racial groups [2]. Results from HHANES suggest that in the 45 to 75 year age group, standardized prevalence of type 2 diabetes mellitus (T2D) was about 16%; 1.3 times higher than in non-Hispanic Whites (12%). In general, one in six Cuban Americans has T2D in the 45 to 74 year age group [3]. The age-specific rate of T2D in Cuban Americans is the highest among any US ethnic group. Risk gradually increases with age among this population: about 8% in the 45 to 54 age group, to about 35% in the 65 to 74 age group [4]. Due to the greater predisposition of Cuban Americans to T2D, it is worthwhile to look at the factors associated with diabetes and insulin resistance.

Accordingly, a variety of peptides referred to as adipocytokines, secreted by white adipose tissue have recently shown to potentially impact glucose and lipid metabolism, consequently contribute to the pathogenesis of metabolic syndrome MetS [5]. Reported associations of hypoadiponectinemia with insulin resistance, obesity, coronary artery disease and dyslipidemia are indicative of adiponectin as a risk factor and potential marker for MetS [6]. Adiponectin predicted MetS independent of insulin sensitivity, body weight, total fat mass, gender and Tanner stage for a study population (N=125) of overweight Latino youths (aged 11.1 ± 1.7 years) with a family history of T2D [7]. A recent meta-analysis [8] of thirteen longitudinal studies (N= 14,598) across ethnicities confirmed the relative risk of T2D declined as plasma adiponectin levels increased per unit μ/mL (RR=0.72(0.67-0.78), α=0.05). Gender and diabetes status were found to be associated with serum adiponectin levels in a Caribbean population, where females with diabetes had significantly lower levels [9]. In an 18 year prospective study of healthy white middle-aged men (N=1074; 45-65 years), hazard ratio (95%CI) of developing T2D with low adiponectin levels and low HDL was 2.63 (1.66-4.15) [10]. A cohort of 32,826 healthy women from the Nurses' Health Study consented and gave blood samples from 1989-1999 and 1059 developed diabetes [11]. After exclusion for missing values, N=1038 cases were matched with 1036 controls [11]. Multivariate models of OR for quintile of adiponectin level and developing T2D were (highest to lowest) 0.12 (0.08-0.17) without adjusting for BMI and 0.17 (0.12-0.25) controlling for BMI ($p<0.001$) [11].

Another biologically active molecule associated with risk for MetS is ghrelin. Ghrelin is a peptide growth hormone synthesized in the stomach and present in the peripheral blood in the acylated and non-acylated forms. Low plasma levels of ghrelin have been associated with MetS and components of MetS: waist circumference and high triglyceride for older adults [12-14]. Lee et al [15] observed an inverse relationship with ghrelin levels and MetS [N=52] with adults on hemodialysis. Primarily Caucasian adults (N=233) ages 23-70, with 5 classes of BMI measured in kg/m^2: underweight to normal (<24.9); overweight (25-29.9); obese I (30-34.9); obese II (35-39.9); and severely obese (> 40) randomly selected from the University of Utah cardiovascular study (N >600,000) were found to have a positive association of plasma ghrelin and BMI [16].

Circulating plasma adiponectin [17, 18] and ghrelin [13] were associated with the development of MetS and insulin resistance; research examining the association may provide insight into the pathways mediating chronic diseases such as chronic heart disease (CHD) and T2D. To date, there have been no reported studies of the association of adiponectin and ghrelin levels with MetS in Cuban American adults. Thus, the primary aim of this study was to determine the association between plasma adiponectin and ghrelin levels with components of MetS in Cuban American adults with and without diabetes. In addition, this study investigated the relationship of MetS components

(CHD risk factors) and their association with high sensitivity C-reactive protein (hs-CRP).

Methods

Study population

The study was conducted with a cross-sectional design using quantitative methods. The target population was Cuban American adults in Broward and Miami-Dade Counties, Florida. The respondents were randomly recruited from two mailing lists of Cuban Americans: identified as those with and without T2D. The address list was purchased from Knowledge Base Marketing Inc., Richardson, TX 75081. Throughout a one-year period, approximately 10,000 letters were sent by systematic selection from the mailing lists (5,000 from list of those with T2D and 5,000 from the list of those without diabetes). The letters described the study in Spanish and English, and were sent along with an invitation flyer containing the investigator's contact information. Three percent (N=300) of the letters were returned due to unknown addresses. From the remaining sent 4% (N=388) responded. Interested respondents were initially interviewed via telephone. The trained interviewers explained the study purpose and inquired preliminary demographics (age, sex, and ethnicity) of the respondents.

To ascertain T2D status, each participant was asked for their age of diagnosis and initial treatment modalities. Only 18 respondents did not qualify for the study: two were not of Cuban origin; nine were less than the required age (< 30 years old); and seven had other chronic illnesses. If a respondent was determined to be eligible, their participation was requested at the Human Nutrition Laboratory at Florida International University (FIU). Participants were instructed to refrain from smoking, consumption of any food and beverages, and any unusual exercise for at least 8 hours prior to their blood collection. Qualified respondents without diabetes were matched for age and gender with participants with T2D.

Florida International University's Institutional Review Board's approved informed consent was presented to eligible respondents in either Spanish or English based on their preference. Signatures were obtained from all participants indicating that they read, understood and agreed to the informed consent form. An additional 19 subjects were excluded due to missing values needed to determine metabolic syndrome (MetS). Data were available for analysis of MetS with adiponectin and ghrelin for 195 participants; whereas, for analysis of MetS with hs-CRP levels data were available for 183 participants. Seven participants reported not having diabetes; but, were reclassified to the group with T2D according to American Diabetes Association (ADA) standards [19]. They were given their laboratory results and referred to their physicians.

Demographics and biometrics

A socio-demographic questionnaire was completed by participants, constituting questions related to age, gender, smoking status, family history of diabetes and family history of heart diseases, cholesterol and anti-inflammatory medication(s). The presence or absence of MetS (binary variable) was formed based upon the National Cholesterol Education Program Adult Treatment Panel III criteria (NCEP-ATP III) [20]. According to NCEP for a classification of MetS three or more of the following coronary heart disease risk factors needed to be present: abdominal obesity (WC > 102 cm for men and > 88 cm for women); hypertension (subjects with systolic BP of ≥ 130 mm Hg and/or diastolic BP of ≥ 85 and/or taking antihypertensive medication); glucose intolerance (fasting plasma glucose of ≥110 mg/dL); hypertriglyceridemia (fasting triglycerides of ≥150 mg/dL)and low high-density lipoprotein cholesterol (<40 mg/dL for men and <50 mg/dL for women). In addition, these MetS factors (coronary heart disease risk factors) were converted to binary variables (based on their presence or absence) for use in analyses with adiponectin and ghrelin.

Blood pressure (BP) was measured twice then averaged in participants at sitting position after a 15-minute rest with a mercury sphygmo-manometer and an adult size cuff. Waist

circumference (WC) was measured at a level midway between the lower rib margin and the iliac crest with a non-stretchable tape all around the body in horizontal position to the nearest 1 cm. Height and weight were measured to the nearest 0.5 cm and 0.1 kg, respectively. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. Ten-year risk of coronary heart disease (CHD) was calculated using the electronic risk assessment tool for estimating 10-year risk of developing CHD [20], where risk factor scores are based upon the following factors: age; gender; total cholesterol; HDL cholesterol; systolic blood pressure; treatment for hypertension; and cigarette smoking. Participants with multiple risk factors were assigned to one of three categories according to 10-year risk stratification for CHD: >20% (high risk), 10–20% (intermediate risk), and <10 % (low risk).

Blood collection and analysis

A 20 ml venous blood was collected from each subject after an overnight fast (at least 8 hours) by a certified phlebotomist using standard laboratory techniques. Blood samples were collected into a vaccutainer Serum Separator Tube (SST) for analysis of lipids and glucose. After complete coagulation (30-45 minutes), the SST was centrifuged at 2500 RPM for 30 minutes. The serum was transferred from the spun SST into 3 labeled plastic tubes: the 1st tube was used for glucose analysis, the 2nd for lipid panel, and the 3rd tube was stored at -70°C to determine serum levels of high-sensitivity C-reactive protein (hs-CRP) by the Immulite method at the Vascular Disease Intervention and Research Laboratory, Edmond, OK. The Immulite assay is a 2-site chemiluminescent enzyme immunometric assay with one monoclonal and one polyclonal anti-CRP antibody. A 1:100 manual dilution of the antibody provides a measurable range of 0.1-500 mg/ L [21]. The intra-assay and the inter-assay coefficients of variation were 3.8% and 7.4%, respectively, and the lower detection limit was 1 ng/mL hs-CRP. Based on abundant epidemiological studies, the Centers for Disease Control and Prevention (CDC) and the American Heart Association (AHA), issued guidelines to use hs-CRP as an adjunct measurement of global risk assessment for cardiovascular disease. In addition, the CDC and AHA indicate a cutoff point of hs-CRP > 3mg/L as a predictor of high relative risk for CVD [22, 23]. Glucose levels were measured by hexokinase enzymatic methods and lipid panel was assayed by enzymatic methods by Laboratory Corporation of America, Miami, FL (LabCorp®). Whole blood for adiponectin was collected into centrifugated tubes containing tripotassium ethylenediamine tetracetate. Adiponectin was measured in the PI's lab using an enzyme-linked immunosorbent assay (ELISA) (Linco Research Inc). The wells of kit for human adiponectin were coated with monoclonal anti-human adiponectin antibodies which captured adiponectin molecules from the sample. Concurrently, the sample adiponectin was bound to the coated antibody sandwiched between a second monoclonal anti-human antibody. After unbound materials were washed from the samples the enzyme streptavindin-horseradish peroxidase conjugate was bound to the immobilized antibodies. The excess free enzyme conjugates were washed and the enzyme activity of horseradish peroxidase in the presence of the substrate 3, 3', and 5.5'-tetramethy-benzidine was measured spectrophotometrically by the increased absorbance at 450nm-590 nm upon product formation. The increase in absorbance is directly proportional to the sample adiponectin captured by the assay. Unknown adiponectin samples were interpolated from a reference curve of an adiponectin standard assayed concomitantly. The projected intra-assay and inter-assay coefficients of variation were7.4% and 8.4%, respectively; and, the sensitivity was 0.78 ng/mL.

Whole blood for Ghrelin analyses was collected in anticoagulant tubes, refrigerator- centrifuged and the plasma was stored at -70°C. A licensed medical technologist detected the level of acylated ghrelin by Enzyme Immunometric Assay (EIA). The Cayman Chemical Inc. kits' reaction wells are coated with monoclonal antibody that is specific to the C-terminal part of ghrelin. An acetylchlinesterase (AchE) conjugate is added after the participants sample allowing the two antibodies to form a sandwich by binding to the acylated ghrelin. The concentration is

determined by measuring the enzyme activity with Ellman's Reagent by intensity with a spectrophotometer at 450 mn.

Data Analysis

All analyses used Statistical Package for the Social Sciences (SPSS) version 17 (SPSS Inc., Chicago, IL, USA). Due to the distributional property of adiponectin a square root transformation was applied. Ghrelin and hs-CPR were log transformed to achieve linearity. Means, standard deviations (SD), frequencies, and percentages were used to describe the participants' characteristics and to assess the distributional properties of the variables. Independent samples t-tests and chi-square tests of independence were conducted to examine differences between participants included in and excluded from the analyses. Bivariate associations among the variables were assessed using Pearson's correlations, controlling for the effect of age, gender and BMI. Analysis of covariance (ANCOVA) was run independently for ghrelin and adiponectin to determine the significance of diabetes status, age and gender. Logistic regression models for adiponectin levels predicting MetS controlling for age, gender and BMI were performed with diabetes status. Mediation and moderation of MetS by diabetes status and gender were tested by logistic regression analyses for adiponectin and ghrelin. The probability of metabolic syndrome was plotted from predicted residuals for adiponectin by diabetic status against deciles of adiponectin. Serum adiponectin levels quartiles by diabetes status and absence or presence of MetS were determined. Stepwise multiple linear regression analyses were performed to determine the relationship of MetS components with adiponectin, ghrelin and hs-CRP. At the 95% confidence interval, two-tailed p-values < 0.05 were considered significant.

Results

Characteristics of the participants were presented as comparison of means by diabetes status (Table 1). A total of 183 subjects provided complete data. The average age among participants with diabetes (n=56) was 60.7±10.8; where the majority were male (55.4%). A significant difference was observed (p=0.015) between calculated BMI of adults with and without diabetes. Individuals without diabetes had significantly higher serum adiponectin (3.4±1.2) versus (2.1±0.8) and ghrelin levels (1.9±.4) versus (1.7±.3) than participants with diabetes, respectively ($p<0.001$). Adults with diabetes had significantly higher of the following attributes: percent of MetS (p=0.001); number of MetS components (p=0.001); average 10-year risk of CHD (p=0.022); WC (105.5±12.5 cm, p=0.001); fasting plasma glucose (166.3±72.0 mg/dL, $p<0.001$) and triglycerides (194.3±113.9 mg/dL, p=0.015) than adults without diabetes.

Table 2 shows the relationships among demographics, and CHD risk factors. Low levels of adiponectin and ghrelin were associated with having T2D. Lower BMI corresponded to higher ghrelin levels. Adiponectin and ghrelin levels were independent of age and gender. Analyses of adiponectin and ghrelin by ANCOVA confirmed no interaction between gender and diabetes status. Adiponectin was positively correlated with the presence of high lipid triglycerides ($r = 0.349$, p=0.005) for adults with diabetes; whereas, there was no significant correlation for those without diabetes (data not shown). Ghrelin level and WC were negatively associated only in participants without diabetes.

High sensitivity-CRP was positively associated with BMI, MetS and number of MetS components. To confirm the correlations, stepwise linear regression analysis of CHD risk factors (MetS components) was conducted to predict hs-CRP levels. Obesity and high fasted plasma glucose explained 14.4% of hs-CRP levels (adjusted $R^2 = 0.144$) ($F=16.3$, $R^2 = 0.144$, $p <0.001$).

Correlation of ghrelin and adiponectin stratified by diabetes status (n=62, with diabetes and n=139, without diabetes) supported the need for logistic models to determine moderation and or mediation. Results of logistic regression models (Table 3) demonstrated that diabetes status moderates the relationship between adiponectin and MetS. A sample weight of factor of 2.12 was

Table 1 Characteristics of the study participants

Variables	Present (n=56)	Absent (n=127)	*P* Value
Age (years)	60.7±10.8	61.2±11.1	0.780
Female %	44.6	65.4	0.010
BMI (Kg/m^2)	31.6±5.8	29.6±5.1	0.015
Waist circumference (cm)	105.5±12.5	98.9±12.1	0.001
Current smoker (%)	18	15	0.062
Systolic BP (mm Hg)	133.3±15.5	131.3±21.0	0.529
Diastolic BP (mm Hg)	81.1±8.6	81.8±10.5	0.631
Fasting plasma glucose (mg/dL)	166.3±72.0	95.8±11.8	<.0,001
Total cholesterol (mg/dL)	198.5±44.7	209.3±44.5	0.133
Triglyceride (mg/dL)	194.3±113.9	152.0±104.4	0.015
HDL cholesterol (mg/dL)	48.8±12.5	56.7±13.3	0.001
Sq Rt [Adiponectin (µ/mL)]	2.1±.8	3.4±1.2	<0.001
Log [hs-CRP (ng/mL)]	.53±.5	.38±.5	0.940
Log [Ghrelin (µ/mL)]	1.7±.3	1.9±.4	0.001
Presence of MetS (%)	30.1	21.3	0.001
Number of MetS components	2.55±1.08	1.66±1.11	<0.001
10 year CHD Risk	9.7±7.2	7.1±6.9	0.022

Data are % or mean ± standard deviation. P <0.05 was considered significant.
BP = blood pressure; HDL= high-density lipoprotein cholesterol; BMI = body mass index; Sq Rt = square root transformed; hs-CRP = high sensitivity C-reactive protein; MetS = metabolic syndrome; CHD = coronary heart disease.

Table 2: Pearson's correlation (rho) of ADPN, ghrelin, MetS, hs-CRP and 10-year risk of CHD of the population

	ADPN	Ghrelin	DM	hs-CRP	# MetS	MetS-y	10y	Age	Sex
ADPN									
Ghrelin	0.132								
DM	- 0.451**	-0.244**							
hs-CRP	-0.114	-0.038	0.141						
# MetS	-0.078	-0.186*	0.351*	0.299*					
MetS -y	-0.109	-0.142	0.289**	0.221*	0.840**				
10 yr	-0.070	0.005	0.169*	0.090	0.256**	0.242**			
Age	0.016	-0.089	-0.021	0.055	0.007	-0.007	0.449**		
Sex	-0.029	-0.055	0.194**	-0.041	0.037	0.084	0.629**	-0.014	
BMI	-0.054	-0.195**	0.180*	0.358**	0.408**	0.293**	-0.050	-0.172*	-0.128

** p<0.05 level; ** p<0.01 level; ADPN=plasma adiponectin; DM= diabetes mellitus status; hs-CRP=high sensitivity C-reactive protein; # MetS = number of MetS components; MetS-y= MetS present; 10yr = 10 year risk for coronary heart disease; BMI = body mass index. Note: correlations of 15% or greater are significant. For biomedical associations, r>0.15 and p<0.05 were considered acceptable.*

Table 3: Logistic regression analysis of the presence of MetS regressed into adiponectin, age, gender and BMI among Cuban American adults with and without diabetes

Independent variables	Beta	SE	P	OR (95% CI)
Adiponectin	0.077	0.030	0.011	1.08 (1.02-1.15)
Diabetes Status (1)*	-0.305	0.528	0.563	0.737 (0.262-2.07)
Adiponectin by Diabetes Status Interaction	-0.158	0.061	0.010	0.854 (0.758-0.963)
Age	0.001	0.014	0.971	1.00 (0.973-1.03)
Gender	0.393	0.303	0.195	1.48 (0.818-2.68)
BMI	0.121	0.028	<0.001	1.13(1.07-1.19)

**Diabetes status (1) = persons with diabetes.*
Notes: χ^2 (6 df) = 52.7. Nagelkerke's Model R^2 = 0.251. Model Classification = 70.3%

Table 4: Quartiles of adiponectin levels in study population with or without metabolic syndrome

Quartiles ADPN		Q1		Q2		Q3		Q4	
	MetS	%	N	%	N	%	N	%	N
without T2D	No	75.0	21	75.6	31	82.8	48	78.7	100
	Yes	25.0	7	24.4	10	17.2	10	21.3	27
with T2D	No	56.3	18	47.6	10	0.0	0	50.0	28
	Yes	43.8	14	52.4	11	100.0	3	50.0	28

Q1, Q2, Q3, Q4 = 1st, 2nd, 3rd and 4th quartile of adiponectin (ADPN). MetS=Metabolic Syndrome.

applied participants with T2D for a logistic regression analysis. This sample weight was used to achieve equal groups in order to determine the probability of MetS associated with adiponectin for each stratum (with and without diabetes) Diabetes status was found to be a mediator for Ghrelin predicting MetS using the four-step proof by logistic regression models (data not shown).

Individuals without T2D who had MetS had a greater percentage (25%) in the lowest quartile for adiponectin (Q1) than those in the highest quartile (21.3%) (Q4) (Table 4). Fifty percent of participants with diabetes but without MetS occupied Q4, while 56.3% occupied Q1. However, 50% of persons with diabetes and MetS occupied the highest quartile while 43.8% occupied Q1.

Figure 1 displays the probability of MetS as a function of adiponectin levels for participants with and without diabetes while holding the remainder of the variables constant at the sample average. For both groups, adiponectin levels under the 20th deciles corresponded with a higher probability of having MetS. Beyond the 20th deciles, the probability of having MetS greatly increased for persons with diabetes as adiponectin levels increased; while, persons without diabetes showed an increased risk below the 40th decile and a relatively unchanged risk for higher levels.

Discussion

This study demonstrated the associations of adiponectin and ghrelin with MetS among Cuban American adults with and without diabetes. The relationship among the components of metabolic syndrome and adiponectin in our study was in accordance with the literature for the stratum without diabetes, only. Adiponectin levels have

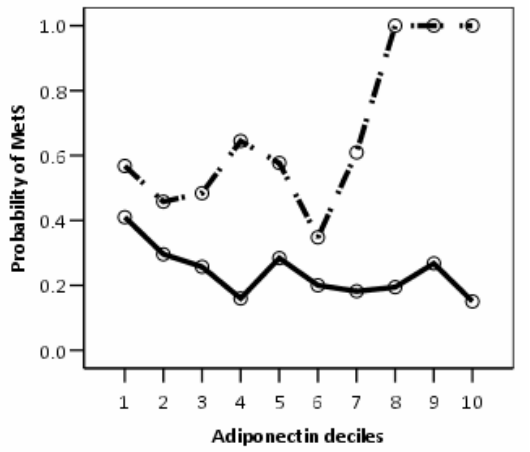

Figure 1: Probability of metabolic syndrome as a function of deciles of serum adiponectin by diabetes status (*Dashed line = respondents with type 2 diabetes; Solid Line = respondents without type 2 diabetes)*

been closely related to different features of MetS such as high triglycerides [24, 25] low HDL [24-26] and decreased insulin sensitivity [18, 27]. The present study indicated that having high adiponectin levels and diabetes were associated with increased risk of MetS. Our results support earlier findings on the relationship between adiponectin and MetS in other study populations such as healthy adults [24; 28-30], elderly [31], and obese individuals [31, 32] where adiponectin at high levels has been reported to suppress inflammation and functions as a protective factor for obesity, T2D and atherosclerosis.

While some recent studies have determined a relationship among MetS, adiponectin levels and diabetes, these studies had smaller sample sizes than our study, and their results were inconsistent. Few studies have shown that hypoadiponectinemia and MetS were associated with individuals with T2D [18, 33]. Although our results agreed with the trend that lower adiponectin levels are associated with diabetes, there were several notable differences. First, no significant differences between genders were found in our study. Second, the persons with diabetes had an increasing probability of MetS with higher adiponectin levels; and third, among persons with (T2D), the diagnosis of MetS is clinically significant. Previous studies have demonstrated that patients who have T2D and also have MetS have a higher risk for CVD than those without MetS [34, 35]. For those with T2D, the presence of MetS has been associated with approximately 5-fold increase in CVD risk. When T2D is first diagnosed, identifying the components of MetS may be a useful prognostic tool for depicting individuals at increased risk for CVD. Metabolic syndrome is also considered a proinflammatory state [36, 37] and measurement of inflammatory markers like hs-CRP and adiponectin might improve the prediction of CHD and T2D in patients with MetS [38]. Therefore, this study enhances the relevance of serum adiponectin as a useful biomarker for the identification of risk for developing MetS in individuals with T2D.

Adiponectin exhibits beneficial anti-diabetic and anti-inflammatory properties by enhancing insulin's ability to stimulate glucose uptake and stimulating anti-atherogenic substances [39, 40]. Hypoadiponectinemia induced by metabolic disorders that are characteristic of MetS, such as insulin resistance, may be a factor in vascular changes [41]. Previous studies have demonstrated that low levels of adiponectin are associated with insulin resistance, and reduced insulin resistance causes adiponectin levels to increase [27, 31]. Thiazolidinediones (TZD) treatment can lead to a uniform increase in adiponectin for individuals with type 2 diabetes. Adiponectin may be partly mediated by TZD-induced changes in insulin levels, which are secondary to the effect of TZD to enhance insulin sensitivity. Therefore, insulin might have an independent effect to modulate adiponectin production from adipocytes [42]. The odds of having MetS exponentially decreased for persons with diabetes as their high serum adiponectin levels decreased. The results suggest the abnormally high adiponectin levels in persons with diabetes may be associated with insulin resistance and high probability for having MetS. In contrast, participants without diabetes followed an approximately 15 degree linear increase in likelihood of having MetS as serum adiponectin levels declined which supports the view of the protective effect of the adipocytokine against MetS.

Studies to date regarding ghrelin and MetS have been single time point and with limited populations: older adults, adults on hemodialysis and Caucasian adults. Our results suggested that ghrelin levels have a limited role in explaining MetS for persons with diabetes. Diabetes status was found to be a mediator (intervening variable) weakening the relationship between ghrelin levels and MetS. For participants without diabetes, our findings support the trend of low ghrelin predicting MetS. Hence, ghrelin may serve as a biomarker for persons without diabetes who at risk for developing MetS. Nevertheless, the dearth of literature regarding ghrelin and MetS, ethnicity and diabetes status warrant further investigation.

Some limitations of this study should be considered. First, insulin resistance was not measured; therefore, the unusually high adiponectin levels that were associated with an increased probability of MetS (for the group with diabetes) were not directly proven to be attributable to insulin resistance. Second, our sample size for the diabetic stratum was weighted to match the non-diabetic stratum for adiponectin to assess the interaction between adiponectin levels and diabetes status with the likelihood of MetS. Third, even though the participants in the present study were Cuban American middle-aged and older adults, they may not represent the target population of Cuban Americans in South Florida. Fourth, given the high rate of obesity among the study participants, a hip to waist ratio may have been more indicative of abdominal obesity than WC. Finally, medications reported for hypertension, T2D or hyperlipidemia were not used to explore interactions with adiponectin, ghrelin or hs-CPP. Several medications have demonstrated to attenuate inflammation-related markers. More specifically TZD increase adiponectin [42] and statins and angiontensin II modulators decrease hs-CRP [43, 44]. However, these effects would tend to bias the results toward null. Further studies are needed to assess the possible interaction T2D medications with adiponectin and ghrelin pharmacokinetics as well as its regulatory controls.

Conclusion

Screening for MetS needs to be made available to the public in order to prevent and manage CHD and T2D. Secondary screenings for persons identified with MetS should include testing for adipocytokines such as ADPN and ghrelin and inflammatory markers such as hs-CRP. Our results suggested high ghrelin levels were protective of MetS (associated with low MetS) regardless of diabetes status; whereas high adiponectin was protective of MetS for those without diabetes only. The results imply these active peptides may be useful in risk assessment for developing CHD and that ADPN and ghrelin may prove important markers for the diagnosis of MetS for Cuban Americans. Further prospective studies assessing adiponectin and ghrelin levels in different populations are warranted to clarify these findings.

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Fatma G. Huffman and Karina Knight-Sepulvelda conceived and designed the study. Michael McLean, Joan Vaccaro and Gustavo G. Zarini collected and analyzed the data. All authors shared responsibility in the intellectual preparation of the manuscript.

References

1. US Census Bureau. 2005-2007 American Community Survey 3-year estimates. Washington, DC: US Census Bureau; 2007.
2. US Census Bureau. Current Population Survey, March 2002, PGP-5. Washington, DC: US Census Bureau; March 2002.
3. Flegal KM, Ezzati TM, Harris MI, Haynes SG, Juarez RZ, Knowler WC, et al. Prevalence of diabetes in Mexican Americans, Cubans, and Puerto Ricans from the Hispanic Health and Nutrition Examination Survey, 1982-1984. Diabetes Care 1991;14(7): 628-638.
4. Harris MI. Epidemiological correlates of NIDDM in Hispanics, Whites and Blacks in the US Population. Diabetes Care 1991;14(7): 639-648.
5. Saltevo J, Vanhala M, Kautiainen H, Kumpusalo E, Laakso M. Association of C-Reactive Protein, interlukin-1 receptor antagonist and adiponectin with metabolic syndrome. Mediators Inflamm 2007;(2007) 1-8.
6. Farvid MS, Ng TWK, Chan DC, Barrett PHR, Watts GF. Association of adiponectin and resistin with adipose tissue compartments, insulin resistance and dyslipidaemia. *Diabetes Obes Metab.* 2005;7:406–413.
7. Shaibi GQ, Cruz ML, Weignenberg MJ, Yoledo-Corral CM, Lane CJ, Kelly LA, et al. Adiponectin independently predicts metabolic syndrome in overweight Latino youth. *J* Clin Endocrinol Metab 2007;92:1809-1813.
8. Lee S, Shin JJ, Ding El, van Dam RM. Adiponectin levels and risk of type 2 diabetes: a systematic review and meta-analysis. *JAMA* 2009;302(2):179-188.
9. Ezenwaka CE, Kallo R. Caribbean female patients; with type 2 diabetes mellitus have lower serum levels of adiponectin than nondiabetic subjects. The Journal of Medicine 2005;63(2):64-69.
10. Koenig W, Khuseyinova N, Baumert J, Meisinger C, Lowel H. Serum concentrations of adiponectin and risk of type 2 diabetes mellitus and coronary heart disease in apparently healthy middle-aged men: Results from the 18-year follow-up of a large cohort from southern Germany. Journal of the American College of Cardiology 2006;48(7):1369-1377.
11. Heidemann C, Sun Q, van Dam RM, Meigs JB, Zhang C, Tworoger SS, et al. Total and High-Molecular-Weight Adiponectin and Resistin in Relation to the Risk for Type 2 Diabetes in Women. Ann Intern Med 2008;149:307-316.
12. Serra-Prat M, Alfarot SR, Palomera E, Casamitjana R, Buquet X, Fernadez-Fernandez C et al. Relationship between ghrelin and the metabolic syndrome in the elderly: a longitudinal population-based study. Clin Endocrinol 2009;70:227-232.
13. Ukkola O, Poykko SM, Antero Kesaniemi Y. Low plasma ghrelin concentration is an indicator of the metabolic syndrome. Ann Med 2006;38:274-279
14. Langenberg C, Bergstrom J, Laughlin GA, Barrett-Connor E. Ghrelin and the metabolic syndrome in older adults. J Clin Endocrinol Metab 2005;90:6448-6453.
15. Lee CC, Lee RP, Subequ YM, Wang CH, Fang TC, Hsu BG. Fasting serum total ghrelin level inversely correlates with metabolic syndrome in hemodialysis patients. Arch Med Research 2008;39:785-790.
16. Monti V, Carlson JJ, Hunt SC, Adams TD. Relationship of ghrelin and leptin hormones with body mass index and waist circumference in a random sample of adults. J Am Diet Assoc 2006;106:822-828.
17. Spranger J, Kroke A, Mohlig M, Bergmann MM, Ristow M, Boeing H et al. Adiponectin and protection against type 2 diabetes mellitus. Lancet 2003;361: 226–228.
18. Mojiminiyi OA, Abdella NA, Al Arouj M, Ben Nakhi A. Adiponectin, insulin resistance and clinical expression of the metabolic syndrome in patients with Type 2 diabetes. Int J Obes 2007;31(2): 213–220.
19. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2008;31: S55-60.
20. National Cholesterol Education Program Expert Panel. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). National Heart, Lung, and Blood Institute, National Institutes of Health, Publication No 02-5215. 2002;3143-3421.
21. Roberts WL, Sedrick R, Moulton L, Spencer A, Rifai N. Evaluation of four automated high-sensitivity C-reactive protein methods: implications for clinical and epidemiological; applications. Clin Chem 2000;46(4):461-468.
22. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO 3rd, Criqui M, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation 2003;107(3):499-511.
23. Cushman M, Arnold AM, Psaty BM, Manolio TA, Kuller LH, Burke GL, et al. C-reactive protein and the 10-year incidence of coronary heart disease in older men and women: the cardiovascular health study. Circulation 2005;112(1):25-31.
24. Ryo M, Nakamura T, Kihara S, Kumada M, Shibazaki S, Takahashi M,, et al. Adiponectin as a biomarker of the metabolic syndrome. Circ J 2004;68(11): 975–981.
25. Matsubara M, Maruoka S, Katayose S. Decreased plasma adiponectin concentrations in women with dyslipidemia. J Clin Endocrinol Metab 2002;87:2764-2769.
26. Valsamakis G, Chetty R, McTernan PG, Al-Daghri NM, Barnett AH, Kumar S. Fasting serum adiponectin concentration is reduced in Indo-Asian subjects

and is related to HDL cholesterol. Diabetes Obes Metab 2003;5:131–135.
27. Tschritter O, Fritsche A, Thamer C, Haap M, Shirkavand F, Rahe S, et al. Plasma adiponectin concentrations predict insulin sensitivity of both glucose and lipid metabolism. Diabetes 2003;52: 239–243.
28. Santaniemi M, Kesaniemi YA, Ukkola O. Low plasma adiponectin concentration is an indicator of the metabolic syndrome. Eur J Endocrinol 2006;155(5): 745–750.
29. Salas-Salvado J, Granada M, Bullo M, Corominas A, Casas P, Foz M. Plasma adiponectin distribution in a Mediterranean population and its association with cardiovascular risk factors and metabolic syndrome. Metabolism 2007;56(11):1486–1492.
30. Kim SM, Cho KH, Park HS. Relationship between plasma adiponectin levels and the metabolic syndrome among Korean people. Endocr J 2006;53(2): 247–254.
31. Choi KM, Lee J, Lee KW, Seo JA, Oh JH, Kim SG, et al. Serum adiponectin concentrations predict the developments of type 2 diabetes and the metabolic syndrome in elderly Koreans. Clin Endocrinol 2004;61(1):75–80.
32. Wang J, Li H, Franco OH, Yu Z, Liu Y, Lin X. Adiponectin and metabolic syndrome in middle-aged and elderly Chinese. Obesity 2008;16(1): 172–178.
33. Hickman IJ, Whitehead JP, Prins JB, Macdonald GA. Raised alanine transaminase and decreased adiponectin are features of the metabolic syndrome in patients with type 2 diabetes. Diabetes Obes Metab 2007;9(3): 438–440.
34. Guzder RN, Gatling W, Mullee MA, Byrne CD. Impact of metabolic syndrome criteria on cardiovascular disease risk in people with newly diagnosed type 2 diabetes. Diabetologia 2006;49(1): 49–55.
35. Bonora E, Targher G, Formentini G, Calcaterra F, Lombardi S, Marini F, et al. The metabolic syndrome is an independent predictor of cardiovascular disease in Type 2 diabetic subjects. Prospective data from the Verona Diabetes Complications Study. Diabet Med 2004;21(1): 52–58.
36. Fernandez-Real JM, Ricart W. Insulin resistance and chronic cardiovascular inflammatory syndrome. Endocr Rev 2003;24(3):278-301.
37. Libby P, Ridker PM. Inflammation and atherosclerosis: role of C-reactive protein in risk assessment. Am J Med 2004;116(Suppl 6A):9S-16S.
38. Huffman FG, Perez Gomez G, Zarini GG. Metabolic Syndrome and high sensitivity C-reactive protein in Cubans Ethn Dis 2009;19:115-120.
39. Ukkola O, Santaniemi M. Adiponectin: a link between excess adiposity and associated comorbidities? J Mol Med 2002;80: 696–702.
40. Kadowaki T and Yamauchi T. Adiponectin and adiponectin receptors. *Endocr Rev* 2005;26(3): 439-451.
41. Matsuzawa Y, Funahashi T, Kihara S, Shimomura I. Adiponectin and metabolic syndrome. Arterioscler Thromb Vasc Biol 2004;24:29–33.
42. Yu JG, Javorschi S, Hevener AL, Kruszynska YT, Norman RA, Sinha M, et al. The effect of thiazolidinediones on plasma adiponectin levels in normal, obese, and type 2 diabetic subjects. Diabetes 2002;51:2968-2974.
43. Jiala I, Stein D, Balis D, Grundy SM, Adams-Huet B, Devaraj S. Effect of hydroxymethyl glutaryl coenzyme A Reductase inhibitor therapy on high sensitive C-reactive protein levels. Circulation 2001;103:1933-1935.
44. Takeda T, Hoshida S, Nishino M, Tanouchi J, Otsu K, Hori M. Relationship between effects of statins, aspirin and angiotensin II modulators on high-sensitive C-reactive protein levels. Atherosclerosis 2003;169:155-158.