Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

Habibeh Yekehtaz 11.30.2020

Ghrelin and predicted 10-year cardiovascular disease risk in the Baltimore Longitudinal Study of Aging

By

Habibeh Yekehtaz
MPH

Executive Master of Public Health

Ghada. N. Farhat, PhD, MPH

Committee Chair

Chee W. Chia, MD

Committee Member

Ghrelin and predicted 10-year cardiovascular disease risk in the Baltimore Longitudinal Study of Aging

By

Habibeh Yekehtaz

MD
Tehran University of Medical Sciences, Iran
2012

Thesis Committee Chair: Ghada. N. Farhat, PhD, MPH

Thesis Committee Member: Chee W. Chia, MD

An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Executive Master of Public Health

2020

Abstract

Ghrelin and predicted 10-year cardiovascular disease risk in the Baltimore Longitudinal Study of Aging

By Habibeh Yekehtaz

Background: Ghrelin, also known as the hunger hormone, was shown to have effects beyond the gastrointestinal system including a potential cardioprotective role. Cardiovascular diseases (CVD) are the leading cause of death among both women and men in the United States. In preventive cardiology settings, two scores are commonly used to predict cardiovascular risk in individuals over a 10-year period, the "Framingham risk score, FRS" and the "Pooled Cohort Equations, PCE". Evidence on the association of ghrelin with cardiovascular risk is limited. The aim of our study was to investigate the association between serum ghrelin level and predicted 10-year cardiovascular risk, using both risk scores, in the Baltimore Longitudinal Study of Aging (BLSA).

Methods: A cross-sectional analysis of 307 participants was performed using BLSA baseline study visits conducted between 2003 and 2007. Both CVD risk scores were calculated for the total study sample based on a group of health factors and laboratory tests that have been indicated to predict developing of cardiovascular events in a 10-year period. Multiple linear regression models were applied to assess associations between baseline 10-year predicted CVD risk and baseline ghrelin level.

Results: In unadjusted and adjusted models, a statistically significant trend for lower ghrelin level was observed with higher cardiovascular risk scores, measured via each of the FRS and PCE formulas (P-value <.0001 for each model). Categorical and continuous measures reflected the same trend.

Conclusions: In conclusion, lower serum level of ghrelin was associated with higher CVD risk in a subsample from the BLSA database. Our findings may provide public health researchers and practitioners new insights into the prevention and control of the cardiovascular events at early stages. Further studies are required to replicate this association.

Ghrelin and predicted 10-year cardiovascular disease risk in the Baltimore Longitudinal Study of Aging

By

Habibeh Yekehtaz

MD
Tehran University of Medical Sciences, Iran
2012

Thesis Committee Chair: Ghada. N. Farhat, PhD, MPH

Thesis Committee Member: Chee W. Chia, MD

A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Executive Master of Public Health

2020

Acknowledgement

I am sincerely thankful to Dr. Ghada Farhat for guiding me on every stage of this study, preparing the draft, analyzing the data, developing inferences, and writing my thesis. Without her support, it would have been very difficult for me to finalize this study.

I am also thankful to Dr. Chee Chia for providing me the opportunity to work on dataset from the BLSA which is conducted by the National Institute on Aging Intramural Research Program. Her advises were quite helpful in getting familiar with the BLSA and assessing the data.

I am also thankful to Dr. Juan Leon and Dr. Laurie Gaydos for helping me in developing my thesis proposal which was the cornerstone of this study.

Through my thesis I have learnt a lot about developing a research hypothesis, literature review, analyzing data, interpreting results, and thinking of implications of study's findings in a real world. I hope this study add a new insight to field of disease prevention regarding the protective effects of ghrelin in the cardiovascular system.

TABLE OF CONTENTS

Introduction

Problem statement

Burden of cardiovascular diseases in the United States

In the United States, cardiovascular diseases (CVD) is the leading cause of death among both women and men (CDC WONDER, 1999–2018). It accounts for 1 in every 4 deaths, with 655,000 Americans dying from CVD annually (Virani et al, 2020) and one individual dying every 36 seconds (CDC WONDER, 1999–2018). Heart diseases disproportionately affect Black individuals compared to Whites, and the incidence and clinical manifestations of CVD differ between these two groups (Van Dyke et al, 2018). Between 2014 to 2015, CVD cost the United States about $219 billion in medicines, health care services, and individuals' productivity loss due to premature mortality (Fryar, Chen, and Li, 2019). Therefore, the high burden of CVD requires innovative preventive and therapeutic interventions to reduce disease morbidity and mortality in the United States.

Predicted 10-year CVD risk

The predicted 10-year CVD risk is a group of health factors and laboratory tests that have been indicated to estimate the chance of individuals without CVD developing cardiovascular events in the next 10 years (Wilson, 2019). Multiple formulas exist for calculating CVD risk, including the Framingham risk score (FRS) (D'Agostino et al, 2008) and the Pooled Cohort Equations (PCE) (Goff DC et al, 2014). The FRS accounts for a broader range of cardiovascular events including coronary heart disease, cerebrovascular events, peripheral artery disease, and heart failure (D'Agostino et al, 2008). On the other hand, the PCE includes hard cardiovascular events including coronary heart disease death, myocardial infarction, and fatal and non-fatal stroke (Goff DC et al,

2014). The FRS is categorized into three levels: Low risk (<10%), Moderate risk (10-20%), and High risk (≥20%) (D'Agostino et al, 2008). The PCE is classified into four groups: Low risk (<5%), Borderline risk (5-7.4%), Intermediate risk (7.5-19.9%), and High risk (≥20%) (Goff DC et al, 2014). These scores identify the probability of developing any enlisting cardiac events during a period of 10 years. For calculation of the CVD risk, both formulas include the following risk factors and laboratory tests: age, sex, total cholesterol, high-density lipoproteins cholesterol, systolic blood pressure, antihypertension medication use, diabetes mellitus status, and current smoking status. The PCE formula adds self-identified race (Black vs. non-Black) to account for increased CVD risk among Black individuals. This formula also includes diastolic blood pressure and low-density lipoprotein cholesterol.

There is also a European CVD risk assessment system that it is based on gender, age, total cholesterol, systolic blood pressure and smoking status. This scoring system is categorized into low and high-risk charts predicting risk of developing CVD in a 10-year period among healthy individuals. In comparison to the FRS and the PCE, the European CVD risk provides only two scores, and does not include hypertension medications and diabetes mellites in the formula. Moreover, it does not include race in calculating CVD risk score, unlike the PCE.

Ghrelin hormone

Ghrelin is a 28-amino acid peptide hormone that was initially extracted from rat stomach in 1999 (Tokudome and Kangawa, 2019). Approximately 60 to 70% of ghrelin is produced and released from the stomach. The remainder of this hormone is secreted from the small intestine with a small amount originating from other organs such as the brain, lungs, pancreas, kidneys, and heart (Tritos and Kokkotou, 2006). Ghrelin acts as a ligand for the growth hormone secretagogue receptor (Wu and Kral, 2004). It is mainly known as the hunger hormone which stimulates food intake and fat

deposition. As a growth hormone releasing peptide, ghrelin regulates various mechanisms throughout the body including pituitary hormone release, energy balance, glucose homeostasis, gastrointestinal motility and excretion, immune function, and bone turnover (Wu and Kral, 2004; Pradhan, Samson, and Sun, 2013). Moreover, this hormone modulates muscle mass improvement in cachectic patients (Pradhan, Samson, and Sun, 2013). Ghrelin also has been implicated in the growth and metastasis of endocrine and non-endocrine tumors (Pradhan, Samson, and Sun, 2013).

There are few studies investigating impacts of ghrelin in healthy individuals. The cardiovascular effects of both endogenous and exogenous ghrelin have been studied in animal models and human subjects. In several human studies, intravenous bolus of ghrelin caused a statistically significant decrease in mean arterial pressure, blood pressure, peripheral vascular resistance, heart rate, and also an increase in stroke volume and cardiac index (Zhang et al, 2010; Khatib, Simkhada, and Gode, 2014). These hemodynamic outcomes are the result of exogenous ghrelin effects on the autonomic nervous system that has led to sympathetic nervous system suppression and parasympathetic system stimulation (Soeki et al, 2008). Similar responses have been concluded in an animal study of ghrelin injection in rats (Soeki et al, 2008).

Although further studies are required to detect mechanistic and clinical connections between CVD risk factors, this hormone has demonstrated active roles in some CVD in the recent decade. A 19-year study from Finland recruiting more than 1000 participants revealed that increased level of serum ghrelin can protect against coronary heart disease (Laurila et al, 2014). This finding was not compatible with results from a former study which showed high plasma ghrelin to be associated with increased carotid artery intima-media thickness in men (Pöykkö et al, 2006). Furthermore, deficiency in ghrelin receptors was found to aggravate vascular inflammation by worsening the instability of atherosclerotic plaques. This observation may support an idea of protective role of

ghrelin against atherosclerosis and its complications (Zhang et al, 2015). Despite these findings, potential protective mechanisms of ghrelin in atherosclerosis require more in vivo research. Thus, identifying a clear mechanism for ghrelin's role in regulating atherosclerosis may facilitate investigations of its therapeutic potential.

Regarding myocardial infarction (MI), it appears that both endogenous and exogenous ghrelin can suppress sympathetic nerve activity in mice following MI, resulting in improved survival (Mao et al, 2012; Schwenke et al, 2008, and 2012). Moreover, one major cause of morbidity after acute MI is ischemic/reperfusion injuries (Mao, Tokudome, Kishimoto, 2014; Ferdinandy et al, 2014). In rat models, ghrelin showed a protective impact against ischemic/reperfusion injuries in many organs such as the liver, intestines, pancreas, kidneys, and spinal cord (Bukowczan et al 2015; Qin et al, 2014; Zhang et al, 2013; Zhang et al, 2012; Rajan et al, 2012). Malignant ventricular arrhythmia and increased cardiac sympathetic nerve activity may occur after MI (Mao et al, 2012). Ghrelin was found to affect vagal nerves and avert increases in sympathetic nerve activity and arrhythmia, findings that may lead to survival improvement in patients with MI (Mao et al, 2013). During the recovery phase of MI, ghrelin may refine cardiac function by stopping excessive sympathetic activity of the heart and reducing epinephrine and norepinephrine (Mao et al, 2013). In rat model of MI, ghrelin treatment demonstrated increased expression of vascular endothelial growth factor which promotes angiogenesis (Yuan et al, 2012). By reducing sympathetic nerve activity and inducing angiogenesis, ghrelin's potential as a therapeutic option for cardiac diseases warrants investigation.

In patients with congestive heart failure (CHF), serum ghrelin level increased in comparison to control group and this hormone decreased after heart transplantation (Lund et al, 2009). This increase was only found in acylated ghrelin and not in total serum ghrelin (Zabarovskaja et al,

2014). In the same study, a reduction in acylation of ghrelin was observed in patients with heart transplantation (Zabarovskaja et al, 2014). Based on this finding, it can be concluded that increased acylation of ghrelin is a physiologic response of the human body to the CHF state (Zabarovskaja et al, 2014). Numerus studies identified other favorable effects of ghrelin in patients with CHF, including cardiac index improvement, decreasing cell apoptosis and myocardial remodeling, cachexia improvement, and reducing sympathetic nerves activities (Baldanzi et al, 2002; Nagaya et al, 2001; Soeki et al, 2014; Zhang et al, 2007; Nagaya et al, 2004).

Cardiac cachexia, a catabolic condition recognized by weight loss and muscle wasting, happens in some patients with end-stage heart failure and can be considered as a risk factor for mortality in patients with CHF (Anker and Coats, 1999). According to a study in rats, ghrelin improved left ventricular dysfunction and reduced the onset of cardiac cachexia (Nagaya et al, 2001). Moreover, in healthy humans, ghrelin caused reduction in cardiac afterload and elevation in cardiac output without any increase in heart rate (Nagaya et al, 2001).

In summary, ghrelin has been shown to have cardioprotective effects in diseases such as heart failure, MI, and fatal arrythmia through a variety of mechanisms including direct effects on cardiovascular cells, modulating growth hormone release, and inhibiting sympathetic nerves activity (Mao et al, 2012; Baldanzi et al, 2002; Nagaya et al, 2001; Soeki et al, 2014; Zhang et al, 2007; Nagaya et al, 2004). These findings suggest that ghrelin as an endogenous hormone can be a promising therapeutic option for CVD. Moreover, in healthy individuals, ghrelin has been shown to decrease mean arterial pressure, blood pressure, peripheral vascular resistance, heart rate, and an increase in stroke volume and cardiac index, suggesting it may have a preventive potential (Zhang et al, 2010; Khatib, Simkhada, Gode, 2014, and Soeki et al, 2008). These cardioprotective

effects of ghrelin set the stage for the current research question on whether serum ghrelin level is associated with CVD risk in individuals free from CVD.

Purpose statement

Hypothesis

The aim of this study is to investigate the association between ghrelin level and the predicted 10-year CVD risk based on two different scoring system, the FRS and the PCE, using data from Baltimore Longitudinal Study of Aging (BLSA). These finding may build a foundation for further investigations into ghrelin's role as a cardiovascular biological marker.

Significance statement

As indicated in the earlier discussion, CVD is the leading cause of death in the United States and disproportionately affects Black individuals. The cardiovascular events and their complications impose extra cost to the U.S. health care system. It appears many causes and risk factors of CVD are preventable, mainly through primary and secondary prevention interventions. Applying a biological marker to predict cardiovascular events in adults who are free of CVD may improve the primary prevention and reduce the burden CVD in the United States. Ghrelin has demonstrated cardioprotective features in both healthy and diseased cardiovascular system. Identifying a potential association between serum ghrelin level and the predicted 10-year CVD risk may create a foundation for further investigations on this endogenous hormone, and assessing it as a potential biomarker to evaluate adults before developing any CVD. Thus, the results of this study may have a preliminary role in enhancing primary prevention in the field of cardiovascular health.

Review of literature

Association of ghrelin with cardiovascular risk

As discussed in the introduction section, ghrelin has cardiovascular protective functions in healthy individuals and injury reduction benefits during cardiovascular events. On the other hand, predicted 10-year CVD risk provides an estimation of developing cardiovascular diseases for adults who are free of CVD. These facts led us to further investigate the role of ghrelin as a cardiovascular biomarker by examining its association with CVD risk.

Despite an extensive literature search, only two studies were found evaluating the possible association of ghrelin and cardiovascular risk. One study was conducted in the cardiology department of the rehabilitation hospital in Romania assessing the link between ghrelin and European cardiovascular risk score (Pop et al, 2015). The other study was from a national hospital in Japan evaluating the effect of ghrelin on cardiovascular parameters in patients with hypertension (Yano et al, 2014).

In 2015, a cross-sectional study in Romania recruited 88 patients with CVD from the cardiology department of the rehabilitation hospital to assess the possible association between serum ghrelin and European cardiovascular risk score. The European cardiovascular risk score was calculated based on a group of health factors and laboratory test consisting of age, gender, total cholesterol, systolic blood pressure, smoking status. In addition, patients were assessed for main cardiovascular risk factors such as obesity, arterial hypertension, diabetes mellitus, metabolic syndrome, smoking and lipid profiles. The study showed a negative association between the European cardiovascular risk score and serum ghrelin concentration (R-square=0.06, P-value=0.015) (Pop et al, 2015).

The second investigation was a cohort study conducted in Japan in 2013. It enrolled 590 hypertensive patients and compared the predictive value of ghrelin with some other biomarkers in the risk of developing cardiovascular events. The biomarkers consisted of high-molecular-weight adiponectin, high-sensitivity C-reactive protein, and plasminogen activator inhibitor 1. The cardiovascular events included coronary artery disease, stroke, congestive heart failure, and sudden death. During a 2.8-year follow up, patients with cardiovascular events had statistically significantly lower serum ghrelin level at baseline compared to those without cardiovascular events ($r = -0.12$, P-value < 0.01). While there were no differences among other biomarkers between patients with and without cardiovascular events. Therefore, the study concluded that ghrelin improved the prediction of CVD in hypertensive patients in contrast to the other examined biomarkers (Yano et al, 2014).

To our knowledge, no investigations have assessed the association of serum ghrelin level with predicted 10-year CVD risk in individuals without CVD. To fill this gap, we conducted the current study to examine this association with two CVD risk calculators, the FRS and PCE, in the context of the BLSA study. The PCE has the advantage of including race as one risk factor for developing CVD. Results from this investigation may prepare a foundation for more studies to consider serum ghrelin level as a predictive biomarker for developing cardiovascular events.

Methods

In order to assess the association between ghrelin level and predicted 10-year CVD risk, a cross-sectional secondary data analysis was performed using data from the BLSA study. To get access to the data, a data transfer agreement was completed and signed between National Institute on Aging Intramural Research Program and Emory University's Rollins School of Public Health. The study was approved by the combined Institutional Review Board of the Johns Hopkins Bayview Medical Center and the National Institute on Aging's Gerontology Research Center.

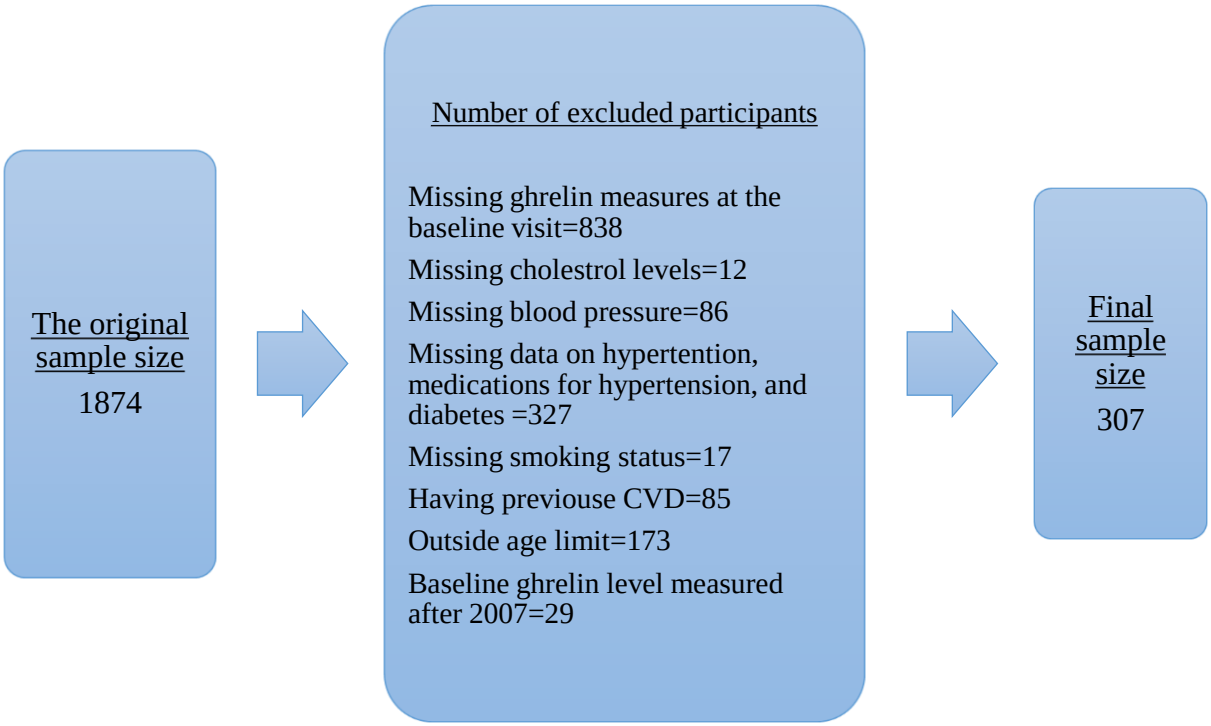
Study population

The BLSA is an ongoing open registration study on human aging established in 1958 and conducted by the NIA's Intramural Research Program. Between 2003 and 2020, the study enrolled 1874 community-based participants aged 22 to 103 years who were free of major chronic conditions and cognitive impairments at the time of enrollment. Participants who enrolled in the study are followed for their entire life, regardless of developing any diseases. These participants have been undergoing comprehensive health screening assessments every 1 to 4 years based on their age (Qiao Y et al, 2019).

The current analysis included a sample of 307 participants and applied a set of exclusion criteria based on age, missing data, and disease history. Because the CVD risk formulas have an age limit (40-79 years old), participants younger than 40 years old and older than 79 years old were excluded from the dataset (n=173). Since ghrelin was one major variable in our study, participants who did not have a baseline ghrelin measurement were excluded (n=838). Participants with missing baseline data on total cholesterol, low-density and high-density lipoprotein cholesterol (n=12),

systolic and diastolic blood pressure (n=86), hypertension, medications for hypertension and diabetes mellites (n=327), and smoking status (n=17) were excluded. Also excluded were participants with previous CVD including angina, MI, CHF, peripheral artery disease, vascular-related procedures (coronary artery bypass grafting or angioplasty), pulmonary disease, kidney disease, peripheral neuropathy, cancer, or lower extremity arthritis pain (n=85). Moreover, due to incompatibility in kits for measuring ghrelin level beyond the 2003 to 2007 period, participants whose baseline ghrelin levels were measured after 2007 were excluded (n=29). This set of exclusion criteria resulted in a final sample size for this analysis of 307 participants. Figure 1 demonstrates the criteria for narrowing down the population of the study.

Figure 1. Study sample, Baltimore Longitudinal Study of Aging, 2003–2007



Data collection

Ghrelin levels, all measurements used in the calculation of the predicted 10-year risks, and data on confounders were collected concurrently from the same baseline study visits during 2003–2007.

Predicted 10-year CVD risk

In the current study, the independent variables were predicted 10-year CV risk that were calculated by applying the FRS and the PCE formulas. As stated earlier, the risk scores show the probability of developing cardiac events during a period of 10 years. For calculation of the CVD risk, both formulas consist of the following risk factors and laboratory tests: age, sex, total cholesterol, high-density lipoprotein cholesterol, systolic blood pressure, antihypertension medication use, diabetes mellitus status, and current smoking status. The PCE formula includes self-identified race (Black vs non-Black) to account for increased CVD risk among Black individuals.

For all participants, the FRS, as a sex-specific score, was calculated using a calculator from the Medscape website (Framingham Risk Score, 2008) and classified into three levels including Low risk (≤10%), Moderate risk (10-20%), and High risk (≥20%). The PCE , which is a sex and race specific score, was quantified based on a calculator from American College of Cardiology website (ASCVD risk estimator plus), and categorized into four groups as follows; Low risk (<5%), Borderline risk (5-7.4%), Intermediate risk (7.5-19.9%), and High risk (≥20%).

Serum ghrelin level

Serum Ghrelin level was the dependent variable. This hormone was obtained from blood samples collected between 7 to 8 in the morning after a 10-hour overnight fasting. Due to the incompatibility of ghrelin measurements across study years, ghrelin data was restricted to the

2003–2007 where the same test kit was used. For this time period, total ghrelin was measured using radioimmunoassay (RIA) and assay kits detected levels in the range of 0.00-1,500.00 pg/ml.

Other study variables

The baseline visits for each participant occurred in different years through 2003 to 2007, but all variables of the current study were collected from participants' baseline visit. At the baseline visit of the BLSA, participants were interviewed about CVD history, current smoking status, and history of medications. Regarding current smoking status, participants were categorized into three groups: current, former, and never smoker.

Height and weight were measured in light clothing using a stadiometer and calibrated scale, respectively. Body mass index (BMI) was assessed as mass in kilograms divided by height in meters squared (Kg/m2). Systolic blood pressure was measured 3 times in each arm with an automated testing device (Colin VP2000/1000) (Martinez et al, 2018). Total cholesterol and high-density lipoprotein cholesterol were measured using blood samples collected between 7 and 8 AM after an overnight fasting. The total cholesterol concentration was evaluated by an enzymatic method (Abbott Laboratories ABA-200 ATC Biochromatic Analyzer, Irving, TX), and the high-density lipoprotein cholesterol was measured by a dextran sulfate-magnesium precipitation procedure (Warnick, Benderson, and Albers, 1982).

Data analysis

In order to characterize the population (N=307), participants were categorized based on the FRS and PCE into 3 and 4 groups, respectively. Baseline characteristics were quantified as mean ± SD for continuous variables or frequencies (proportions) for categorical data. Differences across CVD risk score groups were compared using ANOVA and chi-square test for continuous (age, cholesterol levels, systolic and diastolic blood pressure, BMI, ghrelin level, and predicted 10-year

scores) and categorical variables (sex, race, smoking status, hypertension, and diabetes), respectively. Differences in mean of ghrelin level among different race categories (Black, White, Other) were visualized using a boxplot.

Based on the literature, there are potential correlations among serum ghrelin level and age, sex, BMI. Simple linear regression was used to assess the relationship between ghrelin level and these variables as well as others.

To assess the possible associations between baseline 10-year predicted CVD risk and baseline serum ghrelin level multiple linear regression models were used with adjustment for confounders. Separate models were performed for FRS and PCE scores. Both categorical and continuous forms of the variables were analyzed. For categorical FRS and PCE scores, dummy variables were created and entered in models. The analyses were performed using SAS Enterprise Guide 7.1 (Cary, NC).

Results

Baseline characteristics of population

Tables 1 and 2 describe the baseline characteristics of the population by separate groups based on the FRS (Table 1) and the PCE (Table 2).

The 307 study participants had a mean age of 60.8±9.5 years and were 54.0%women and 30.9% Black. The predicted FRS among all participants was 11.7±9.6, and the PCE was 10.5±11.7. The mean ghrelin level was 256.7±142.3 pg/ml (Table 1 and 2).

For the FRS, a statistically significant trend for lower ghrelin level was observed with higher FRS groups (p-value <.0001) (Table 1). Similarly, for PCE, a statistically significant trend for lower ghrelin level was observed with higher PCE groups (p-value <.0001) (Table 2).

According to the boxplot in figure 3, mean of ghrelin level was slightly lower in Black compared to White individuals, and this difference was statistically significant (p-value= 0.0031).

Assessing for confounding factors

Simple linear regression models assessing associations of ghrelin with study variables revealed several statistically significantly associations. Age, sex, systolic blood pressure, and BMI showed a negative association with ghrelin level, while high-density lipoprotein cholesterol had a positive association with the hormone level (Table 3). Because the CVD risk scores include in their respective formulas many of these variables, our final models were adjusted only for BMI.

Association between the predicted 10-Year CVD risk and serum ghrelin level

FRS models:

In adjusted multiple linear regression models, a statistically significant trend for lower ghrelin levels with higher FRS categories was observed. Ghrelin level was shown to decrease by 65 pg/ml in those with Moderate FRS score compared to those in the Low risk score (Reference Group), and by 91 pg/ml in those with High FRS score compared to those in the Low risk score, and these differences were statistically significant (P-value <.0001) (Table 4). A model that used the continuous FRS score yielded similar findings: FRS was negatively associated with ghrelin level (parameter estimate= -4.19, p-value= <.0001). (Table 5).

PCE models:

In adjusted multiple linear regression models, a statistically significant trend for lower ghrelin levels with higher PCE categories was detected. Ghrelin level decreased by 37 pg/ml in those with Borderline PCE score compared to those in the Low risk score (Reference Group), and by 77 pg/ml in those with Intermediate PCE score compared to those in the Low risk score, and by 103 pg/ml in those with High PCE score compared to those in the Low risk score , and these differences were statistically significant (P-value <.0001) (Table 4). A model that used the continuous PCE score showed similar findings: PCE was negatively associated with ghrelin level (parameter estimate= -2.99, p-value= <.0001). (Table 5).

By comparing the results for the FRS and PCE models, it appears the FRS showed stronger associations potentially due to the further accounting for race in the PCE models.

Table 1. Baseline characteristics of the study population by 10-year Framingham Risk Score (FRS) categories, Baltimore Longitudinal Study of Aging, 2003–2007

Characteristics	Overall	FRS-Low	FRS-Moderate	FRS-High	P-Value*
	N=307	n=172	n=63	n=72	
Age, mean ± SD**	60.8±9.5	55.9±7.9	63.6±7.2	69.8±7.0	<.0001
Female %	54.0%	77.9%	46.0%	4.1%	<.0001
Black race %	30.9%	32.5%	44.4%	15.2%	0.007
Current smoker %	4.5%	1.7%	3.1%	12.5%	<.0001
Total cholesterol (mg/dl), mean ± SD	195.3±37.4	196.4±32.6	208.0±42.8	181.7±39.3	0.0002
High-density lipoproteins (mg/dl), mean ± SD	58.8±18.0	64.7±17.0	54.9±15.2	47.9±16.9	<.0001
Low-density lipoproteins (mg/dl), mean ± SD	115.4±33.5	112.9±29.9	130.6±39.5	108.2±32.5	0.0002
Systolic blood pressure (mmHg), mean ± SD	119.9±14.6	113.5±11.1	126.8±12.9	129.4±15.8	<.0001
Diastolic blood pressure (mmHg), mean ± SD	68.6±8.5	66.9±7.7	72.0±8.2	69.7±9.5	0.0001
Body mass index (Kg/m^2), mean ± SD	27.5±4.8	26.7±4.9	28.4±4.6	28.5±4.2	0.006
Hypertension %	39.4%	26.1%	50.7%	61.1%	<.0001
Diabetes mellites %	14.0%	2.9%	11.11%	43.0%	<.0001
Ghrelin, mean ± SD (pg/ml)	256.7±142.3	297.1±162.2	219.8±99.9	192.3±76.4	<.0001
Framingham risk score (FRS)	11.7±9.6	4.6±2.0	13.6±2.7	27.2±3.3	

*Obtained from ANOVA for continuous variables and chi-square test for categorical data

**Standard deviation

Table 2. Baseline characteristics of the study population by 10-year Pooled Cohort Equations (PCE) categories, Baltimore Longitudinal Study of Aging, 2003–2007

Characteristics	Overall	PCE-Low	PCE-Borderline	PCE-Intermediate	PCE-High	P-value*
	N=307	n=136	n=33	n=88	n=50	
Age, mean ± SD**	60.8±9.5	53.6±6.4	61.0±4.9	64.9±6.8	72.9±5.5	<.0001
Female %	54.0%	80.1%	60.6%	37.5%	8.0%	<.0001
Black race %	30.9%	27.9%	39.3%	43.1%	12.0%	0.008
Current smoker %	4.5%	2.2%	0	7.9%	8.0%	<.0001
Total cholesterol (mg/dl), mean ± SD	195.3±37.4	197.0±33.5	201.5±34.8	198.6±41.1	180.7±39.9	0.023
High-density lipoproteins (mg/dl), mean ± SD	58.8±18.0	64.4±16.6	58.2±15.0	56.2±19.0	48.5±16.6	<.0001
Low-density lipoproteins (mg/dl), mean ± SD	115.4±33.5	113.8±31.4	121.3±28.6	121±38.7	106.3±30.8	0.058
Systolic blood pressure (mmHg), mean ± SD	119.9±14.6	113.2±11.5	118.7±12.1	124.0±13.5	131.9±15.9	<.0001
Diastolic blood pressure (mmHg), mean ± SD	68.6±8.5	67.4±7.1	68.9±9.4	69.9±8.9	69.6±10.2	0.138
Body mass index (Kg/m^2), mean ± SD	27.5±4.8	26.9±5.0	28.5±4.7	27.5±4.3	28.4±4.8	0.148
Hypertension %	39.4%	24.2%	39.3%	50.0%	62.0%	<.0001
Diabetes mellites %	14.0%	2.2%	9.0%	15.9%	46.0%	<.0001
Ghrelin, mean ± SD (pg/ml)	256.7±142.3	304.5±171.4	254.6±113.7	222.1±102.0	188.9±73.5	<.0001
Pooled Cohort Equations (PCE)	10.5±11.7	2.1±1.2	6.1±0.7	12.7±3.8	32.2±11.4	

*Obtained from ANOVA for continuous variables and chi-square test for categorical data

**Standard deviation

Figure 3. Boxplot for ghrelin level by race, Baltimore Longitudinal Study of Aging (BLSA), 2003–2007

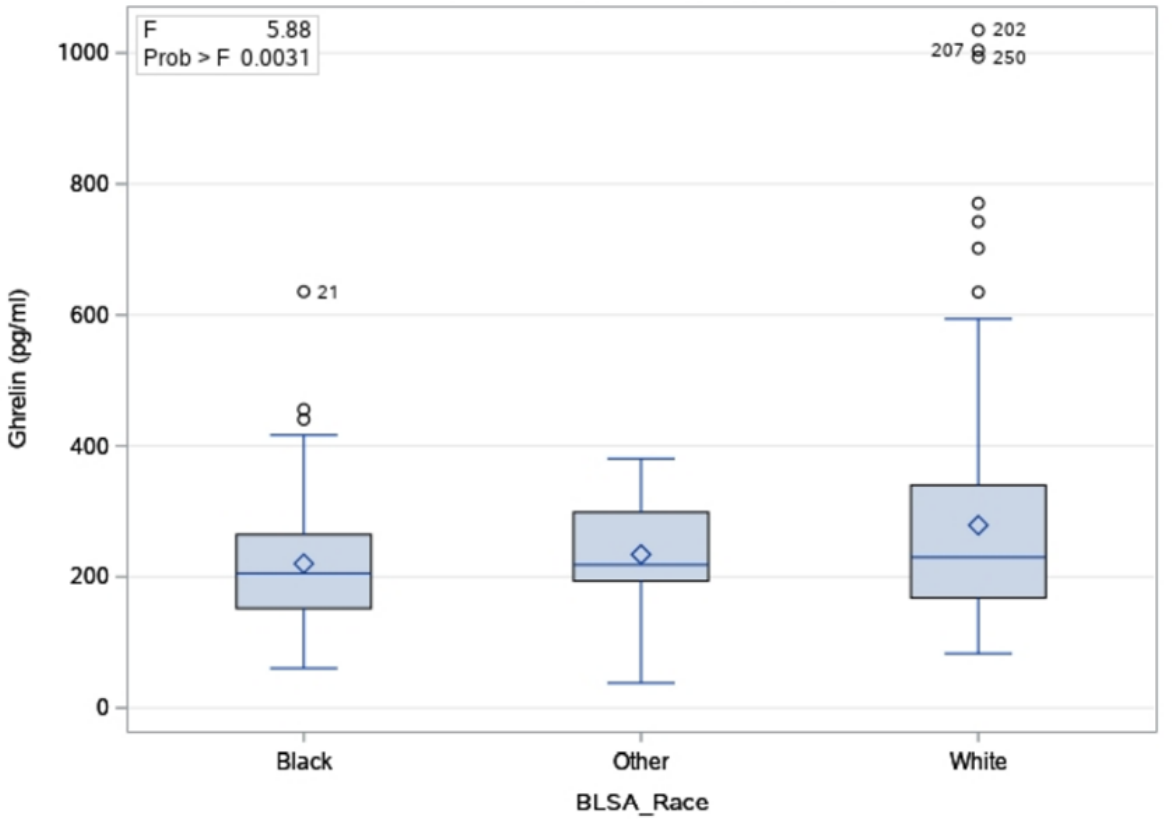


Table 3. Associations of study variables with ghrelin level using simple linear regression, Baltimore Longitudinal Study of Aging, 2003–2007

Variables	Parameter estimates	R-square	P-value
Age	-2.3	0.02	0.005
Sex	-87.7	0.09	<.0001
Systolic blood pressure (mmHg)	-2.4	0.06	<.0001
High-density lipoprotein	2.3	0.09	<.0001
Body mass index (kg/cm^2)	-8.8	0.09	<.0001

Table 4. Adjusted* associations of 10-year predicted cardiovascular risk scores (categorical form) with ghrelin level, Baltimore Longitudinal Study of Aging, 2003–2007

Models (Categorical CVD risk score)	Parameter estimates	R-square	P-value
Framingham Risk Score (FRS) Model	Moderate FRS = -65.13	0.16	<.0001
	High FRS = -91.39 Low FRS = Reference group		
Pooled Cohort Equations (PCE) Model	Borderline PCE = -37.09	0.17	<.0001
	Intermediate PCE = -77.13		
	High PCE = -103.42 Low PCE = Reference group		

* Results obtained from multiple linear regression models adjusted for body mass index

Table 5. Adjusted* associations of 10-year predicted cardiovascular risk scores (continuous form) with ghrelin level, Baltimore Longitudinal Study of Aging, 2003–2007

Models (Continuous CVD risk score)	Parameter estimates	R-square	P-value
Model 1	-4.19	0.16	<.0001
Model 2	-2.99	0.14	<.0001

*Results obtained from multiple linear regression models adjusted for body mass index

Discussion

Summary of study

As stated earlier, ghrelin has demonstrated promising cardioprotective effects in both healthy and diseased cardiovascular states. This secondary data analysis tested the hypothesis that serum ghrelin level is associated with 10-year CVD risk scores, assessed separately using the FRS and the PCE, in the BLSA study. We observed inverse associations between ghrelin and both risk scores (FRS and PCE), measured categorically and continuously, which were statistically significant.

Discussion of key results

The current study demonstrated negative associations between predicted 10-year CVD risk (FRS and PCE) and serum ghrelin level in a subsample of the BLSA database. To our knowledge, no investigation has assessed the association of ghrelin with cardiovascular risk scores, particularly in healthy individuals. Results of the current study can be considered a preliminary exploration of ghrelin as a potential cardiovascular biomarker.

As stated earlier, in a cross-sectional study on Romania, Pop et al. studied 88 patients with history of CVD to assess the possible association between serum ghrelin and European cardiovascular risk score. Their findings showed a negative association between the risk score and serum ghrelin concentration (R-square=0.06, P-value=0.015) (Pop et al, 2015). In Japan, a cohort study was conducted to compare the predictive value of ghrelin with some other biomarkers in the risk of developing cardiovascular events in 590 hypertensive patients. During a 2.8-year follow up, patients with cardiovascular events had statistically significantly lower serum ghrelin level at baseline compared to those without cardiovascular events ($r = -0.12$, P-value < 0.01) (Yano et al,

2014). While in the current study, 307 participants free from CVD were assessed for finding the association between predicted 10-year CVD risk (FRS and PCE) and serum ghrelin level. Compared to the previous studies, main differences of our study include selecting participants without history of CVD and using two scoring system for the cardiovascular risk. In addition, the PCE adds race to the formula as a risk factor for developing CVD which has not been studied previously.

Moreover, we assessed the association of ghrelin level with several variables. Statistically significant negative associations were observed with age, sex (ghrelin was higher among males), systolic blood pressure, and BMI, and positive associations were observed with high-density lipoprotein cholesterol. According to the literature, age, sex, and BMI affect ghrelin level. With aging, a decline in ghrelin level and impairment in endogenous hormone signaling were observed (Amitani M et al, 2017). Ghrelin level was also found to be significantly higher in females comparing to males (Abu-Farha M et al, 2014). Moreover, ghrelin level was found to be inversely associated with the BMI (Monti et al. 2006). Results of the current study were consistent with the previous investigations.

As stated earlier, cardiovascular diseases affect Black individuals disproportionately, and in the analysis of current study "race" was included as a risk factor for developing cardiovascular events by applying the PCE formula. Race was not consistently adjusted for in other investigations, such as the study by Pop et al. which used the European CVD risk score (Pop et al, 2015).

Limitations and strengths

One limitation of the study was that BLSA participants were relatively healthy compared to the general population. Therefore, the magnitude of the associations between the dependent and independent variables of our study may not be generalizable to the general population. Moreover,

due to the variation in sampling kits for ghrelin, there was a discrepancy among measurements of this hormone. This precluded us from pooling results that used different kits and resulted in smaller sample sizes for the analysis.

As a strength of our study, the extensive and accurate data from the BLSA database should be acknowledged. There were numerus risk factors and laboratory tests that were required for conducting the current analysis. These parameters were accessible through this database.

Implications

Finding of this study may create a foundation for further investigations on ghrelin, as an endogenous hormone, and evaluating it as a potential biological marker to evaluate adults before developing any CVD. Moreover, these results can have a preliminary role in enhancing primary prevention in the field of cardiovascular health.

Recommendations

As a main recommendation, conducting longitudinal studies for evaluating the association of ghrelin with future cardiovascular events among disease-free individuals may improve understanding of ghrelin's role as a potential cardiovascular biomarker. Additionally, replicating the results with studies including larger population size may reveal the strength of association between ghrelin and CVD risk.

Since the BLSA participants were mostly healthy adults, replication of our study in individuals with more similarities to the general population may provide more precise and practical results. Confirming ghrelin's role as a potential biological marker for cardiovascular health may ultimately reduce morbidity and mortality of CVD among Americans.

Conclusion

In conclusion, the higher CVD risk is inversely associated with lower serum level of ghrelin. Our findings may provide public health researchers and practitioners new insights into the prevention and control of the cardiovascular events at early stages. Further studies are required to confirm this association.

References

Abu-Farha M et al. (2014). *Gender differences in ghrelin association with cardiometabolic risk factors in Arab population*. International Journal of Endocrinology. Doi: 10.1155/2014/730472

Amitani M et al. (2017). *The role of ghrelin and ghrelin signaling in aging*. International Journal of Molecular Science; 18(7), 1511. Doi:10.3390/ijms18071511.

Anker SD and Coats AJS. (1999). *Cardiac cachexia: a syndrome with impaired survival and immune and neuroendocrine activation*. Chest; 115, 836–847.

ASCVD risk estimator plus. Retrieved from http://tools.acc.org/ASCVD-Risk-Estimator- Plus/#!/calculate/estimate/

Baldanzi G, et al. (2002). *Ghrelin and des-acyl ghrelin inhibit cell death in cardiomyocytes and endothelial cells through ERK1/2 and PI 3-kinase/AKT*. Journal of Cell Biology; 159, 1029–1037.

Baltimore Longitudinal Study of Aging (BLSA). Retrieved from https://www.blsa.nih.gov

Bukowczan J, et al. (2015). *Therapeutic effect of ghrelin in the course of ischemia/reperfusion-induced acute pancreatitis*. Current Pharmaceutical Design; 21, 2284–2290.

Centers for Disease Control and Prevention. *Underlying Cause of Death, 1999–2018*. CDC WONDER Online Database. Atlanta, GA: Centers for Disease Control and Prevention; 2018. Accessed March 12, 2020.

D'Agostino RB Sr, et al. (2008). *General cardiovascular risk profile for use in primary care: the Framingham Heart Study*. Circulation; 117, 743–753.

Ferdinandy P, et al. (2014). *Interaction of risk factors, comorbidities, and comedications with ischemia/reperfusion injury and cardioprotection by preconditioning, postconditioning, and remote conditioning*. Pharmacological Reviews; 66, 1142–1174.

Framingham Risk Score (2008). Retrieved from https://reference.medscape.com/calculator/252/framingham-risk-score-2008

Fryar C.D, Chen T.C, Li X. (2019). *Prevalence of uncontrolled risk factors for cardiovascular disease: United States, 1999–2010*. NCHS data brief, no. 103. Hyattsville, MD: National Center for Health Statistics; 2012. Accessed May 9, 2019.

Goff DC Jr, et al. (2014). *American College of Cardiology/ American Heart Association Task Force on Practice G. 2013 ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines*. Circulation; 129, S49–S73.

Khatib MN, Simkhada P, Gode D. (2014). *Cardioprotective effects of ghrelin in heart failure: from gut to heart*. Heart views, 15(3): 74–76. Doi: 10.4103/1995-705X.144792.

Laurila M, et al. (2014). *High plasma ghrelin protects from coronary heart disease and Leu72Leu polymorphism of ghrelin gene from cancer in healthy adults during the 19 years follow-up study*. Peptides; 61, 122–129.

Lund LH, et al. (2009) *Ghrelin resistance occurs in severe heart failure and resolves after heart transplantation*. European Journal of Heart Failure; 11, 789–794.

Mao Y, Tokudome T, Kishimoto I. (2014). *The cardiovascular action of hexarelin*. Journal of Geriatric Cardiology; 11, 253–258.

Mao Y, et al. (2013). *Excessive sympathoactivation and deteriorated heart function after myocardial infarction in male ghrelin knockout mice*. Endocrinology; 154, 1854–63. [PubMed: 23515286]

Mao Y, et al. (2012). *Ghrelin prevents incidence of malignant arrhythmia after acute myocardial infarction through vagal afferent nerves*. Endocrinology; 153, 3426–34. [PubMed: 22535766]

Martinez-Amezcua P, et al. (2018). *Fatigability and functional performance among older adults with low-normal ankle-brachial index: cross-sectional findings from the Baltimore Longitudinal Study of Aging*. Atherosclerosis; 272, 200–206.

Monti V, Carlson J, Hunt S.C, Adams T.D. (2006). *Relationship of ghrelin and leptin hormones with body mass index and waist circumference in a random sample of adults*. Journal of the American Dietetic Association; 106(6), 822-8. Doi: 10.1016/j.jada.2006.03.015.

Nagaya N, et al. (2004). *Effects of ghrelin administration on left ventricular function, exercise capacity, and muscle wasting in patients with chronic heart failure*. Circulation; 110, 3674–3679.

Nagaya N, et al. (2001). *Chronic administration of ghrelin improves left ventricular dysfunction and attenuates development of cardiac cachexia in rats with heart failure*. Circulation; 104, 1430–1435.

Nagaya N, et al. (2001). *Hemodynamic and hormonal effects of human ghrelin in healthy volunteers*. American Journal of Physiology (Regulatory, Integrative and Comparative Physiology). 2001; 280, R1483–R1487.

Pop D et al. (2015). Serum ghrelin level is associated with cardiovascular risk score. Romanian Journal of Internal Medicine; 53(2), 140–145.

Pöykkö SM, et al. (2006). *Plasma ghrelin concentrations are positively associated with carotid artery atherosclerosis in males*. Journal of Internal Medicine; 260, 43–52.

Pradhan G, Samson S.L, and Sun Y. (2013). *Ghrelin: much more than a hunger hormone*. Current Opinion in Clinical Nutrition & Metabolic Care; 16(6), 619–624. Doi:10.1097/MCO.

Qiao Y et al. (2019). *Association between cardiovascular risk and perceived fatigability in mid-to-late life*. Journal of the American Heart Association. Doi: 10.1161/JAHA.119.013049.

Qin Y, et al. (2014). *Ghrelin contributes to protection of hepatocellular injury induced by ischemia/reperfusion*. Liver International;34, 567–575.

Rajan D, et al. (2012). *Human ghrelin protects animals from renal ischemia-reperfusion injury through the vagus nerve*. Surgery; 151, 37–47.

Schwenke D.O., et al. (2012). *One dose of ghrelin prevents the acute and sustained increase in cardiac sympathetic tone after myocardial infarction*. Endocrinology; 153, 2436–2443.

Schwenke D.O., et al. (2008). *Early ghrelin treatment after myocardial infarction prevents an increase in cardiac sympathetic tone and reduces mortality*. Endocrinology; 149, 5172–5176.

SCORE risk chart. European society of cardiology. Retrieved from https://www.escardio.org/Education/Practice-Tools/CVD-prevention-toolbox/SCORE-Risk-Charts

Soeki T, et al. (2014). *Effect of ghrelin on autonomic activity in healthy volunteers*. Peptides; 62, 1–5.

Soeki T, et al. (2008). *Ghrelin suppresses cardiac sympathetic activity and prevents early left ventricular remodeling in rats with myocardial infarction*. American Journal of Physiology: Heart and Circulatory Physiology; 294: H426–H432. Doi:10.1152/ajpheart.00643.

Tokudome T and Kangawa K. (2019). *Physiological significance of ghrelin in the cardiovascular system*. Doi: 10.2183/pjab.95.032

Tritos N.A and Kokkotou E.G. (2006). *The physiology and potential clinical applications of ghrelin, a novel peptide hormone*. Mayo Clinic Proceedings; 81(5), 653-660.

Van Dyke M, et al. (2018). *Heart Disease Death Rates among Blacks and Whites Aged ≥35 Years — United States, 1968–2015*. Surveillance Summaries, 67(5); 1–11.

Virani SS, et al. (2020). *Heart disease and stroke statistics*—2020 update: a report from the American Heart Association. Circulation;141(9), e139–e596.

Warnick GR, Benderson J, Albers JJ. (1982). *Dextran sulfate-Mg2+ precipitation procedure for quantitation of high-density-lipoprotein cholesterol*. Clinical Chemistry;28, 1379–1388.

Wilson PW. (2019). *Cardiovascular disease risk assessment for primary prevention*. UpToDate. Retrieved from https://www.uptodate.com/contents/cardiovascular-disease-risk-assessment-for-primary-prevention-our- approach

Wu J.T and Kral J.G. (2004). *Ghrelin, integrative neuroendocrine peptide in health and disease*. Annals of Surgery; 239(4), 464-474. DOI: 10.1097/01.sla.0000118561.54919.61.

Yano Y et al. (2014). *Circulating des-acyl ghrelin improves cardiovascular risk prediction in older hypertensive patients*. American Journal of Hypertension; 27(5), 727–733. Doi:10.1093/ajh/hpt232.

Yuan MJ, et al. (2012). *Myocardial angiogenesis after chronic ghrelin treatment in a rat myocardial infarction model*. Regulatory peptides; 179, 39–42. [PubMed: 22960289]

Zabarovskaja S, et al. (2014). *Acylation of ghrelin is increased in heart failure and decreases post heart transplantation*. Scandinavian Cardiovascular Journal; 48, 343–348.

Zhang G et al. (2010). *Ghrelin and cardiovascular diseases*. Current cardiology reviews, 6(1): 62–70. Doi: 10.2174/157340310790231662.

Zhang H, et al. (2013). *Ghrelin attenuates intestinal ischemia/reperfusion injury in mice by activating the mTOR signaling pathway*. International Journal of Molecular Medicine; 32, 851–859.

Zhang M, et al. (2015). *Ghrelin receptor deficiency aggravates atherosclerotic plaque instability and vascular inflammation*. Frontiers in Bioscience (Landmark Ed); 20, 604–613.

Zhang M, et al. (2007). *Effect of exogenous ghrelin on cell differentiation antigen 40 expression in endothelial cells*. Acta Biochimica et Biophysica Sinica (Shanghai);39, 974–981.

Zhang Q, et al. (2012). *Acute effect of Ghrelin on ischemia/reperfusion injury in the rat spinal cord*. International Journal of Molecular Sciences; 13, 9864–9876.