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# Physiological and Psychological Stressors Associated with Glucose Metabolism in the Boston Puerto Rican Health Study

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# PHYSIOLOGICAL AND PSYCHOLOGICAL STRESSORS ASSOCIATED WITH GLUCOSE METABOLISM IN THE BOSTON PUERTO RICAN HEALTH STUDY

A Dissertation Presented

By

ANDREA A. LOPEZ-CEPERO

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# ABSTRACT

**Background:** Puerto Ricans experience high prevalence of type 2 diabetes (diabetes). Stress is a risk factor for diabetes. The allostatic load (AL) model explains how stress influences disease through a chain of physiological changes. Puerto Ricans experience psychological and physiological (obesity and high glycemic load (GL)) stressors linked with diabetes, yet how these stressors impact the AL chain and how their interplay affects glucose metabolism remains unknown.

**Methods**: Using data from the Boston Puerto Rican Health Study, this thesis sought to examine: 1) the relationship between GL and primary AL markers, 2) the interaction between perceived stress and GL on HbA1c, and if primary AL markers mediate this interaction, and 3) the interaction between change in weight and in perceived stress on HbA1c.

**Results**: 1) GL change over 2 years was associated with increases in primary AL markers in women. 2) Women with high perceived stress and high GL had higher HbA1c and primary AL markers did not mediate this interaction. 3) In women, there was an interaction between change in weight and perceived stress on HbA1c over 2 years, with the effect of weight change on HbA1c being greater with increases in perceived stress. None of these associations were observed in men.

**Conclusion**: This study partially confirms the AL model in Puerto Rican women but not in men. It provides data to inform intervention targets to prevent and manage diabetes in Puerto Rican women and identifies women at high risk of diabetes in this minority group.

# TABLE OF CONTENTS

# LIST OF TABLES

## LIST OF FIGURES

## PREFACE

Chapter II of this dissertation is under preparation as:

Lopez-Cepero A, Rosal MC, Frisard C, Person S, Ockene I, Tucker K. Glycemic load is associated with primary stress markers of allostatic load in Puerto Rican women.

Chapter III of this dissertation is under preparation as:

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Chapter IV of this dissertation is under preparation as:

Lopez-Cepero A, Frisard C, Person S, Ockene I, Tucker K, Rosal MC. Changes in perceived stress moderate the association between weight change and HbA1c change in Puerto Rican women.

# CHAPTER I:
# INTRODUCTION

## Epidemiology of Type 2 Diabetes in the United States

The prevalence of type 2 diabetes (referred to as diabetes) has been steadily increasing for almost two decades in the United States (U.S.).[1,2] Diabetes is a serious health condition accompanied by numerous complications, including cardiovascular disease.[3] Diabetes is mainly caused by a rise in blood glucose and its pre-clinical period may last years, making diabetes a preventable disease. Disparities in diabetes have been documented for individuals of ethnic minorities and women.[4–9] In particular, Puerto Ricans experience one of the highest prevalence of diabetes compared to other Latino and non-Latino White groups.[10,11] It is estimated that one in five Puerto Rican women and one in six Puerto Rican men residing in the mainland U.S. have diabetes.[10] With 300,000 Puerto Ricans living in Massachusetts (MA), this group comprises the largest Latino group in the state.[12] Thus, given the individual and societal cost of diabetes,[13,14] and the large population of Puerto Ricans residing in MA, efforts to understand factors that influence diabetes risk in this group are warranted.

## Allostatic Load is a Model for Diabetes Development

The allostatic load (AL) model provides a framework to understand how stress may influence the development of chronic diseases, such as diabetes.[15] The AL model explains how the body's biological regulatory systems respond to physiological and psychological stress. AL is defined as "the wear and tear of regulatory systems due to constant or chronic exposure to stress".[16] AL is hypothesized to be comprised of a chain

of cause and effect, that are characterized by dysregulation of 1) primary AL markers, 2) secondary AL markers, and 3) tertiary AL markers (Figure 1). When there is stress, primary AL markers are secreted by the hypothalamic-pituitary adrenal axis (HPA) and the sympathetic nervous system (SNS). Some commonly used biomarkers to represent activation of these systems are cortisol (HPA activity), DHEA-S (HPA antagonist), epinephrine (SNS activity), and norepinephrine (SNS activity).[15,16] Activation of the immune system, measured by markers like C-Reactive Protein, also occurs during this initial response.[15,16] Chronic dysregulation of these primary AL markers due to constant or chronic stress leads to dysregulation of secondary AL markers, some of which are considered risks factors for diabetes: adiposity, blood pressure, blood lipids and blood glucose.[15,16] Lastly, with chronic dysregulation of these secondary AL markers, chronic diseases such as diabetes develop (tertiary AL markers).

Although AL is conceptualized as a cascade of events, the majority of the studies have assessed AL with a composite measure, using a single score that represents a summary of primary, secondary and tertiary markers of AL. While this methodology has helped understand associations between AL and many social stressors and diseases,[17–24] it hinders the ability to examine causal patterns between primary and secondary biomarkers as well as complex causal associations among biomarkers within the same stage.[25] Thus, research that evaluates AL in its stages (primary markers vs. secondary markers, vs. tertiary markers), in response to physiological and psychological stress, is needed to truly understand disease development. Additionally, the role of sex in the AL model has not been extensively studied. Research studies show that women report greater distress than

men after a stressful life event.[26,27] Thus, given the existing sex differences in diabetes prevalence[8,9] and in the perception of stressful events,[26,27] it is imperative to study the role of sex in AL.

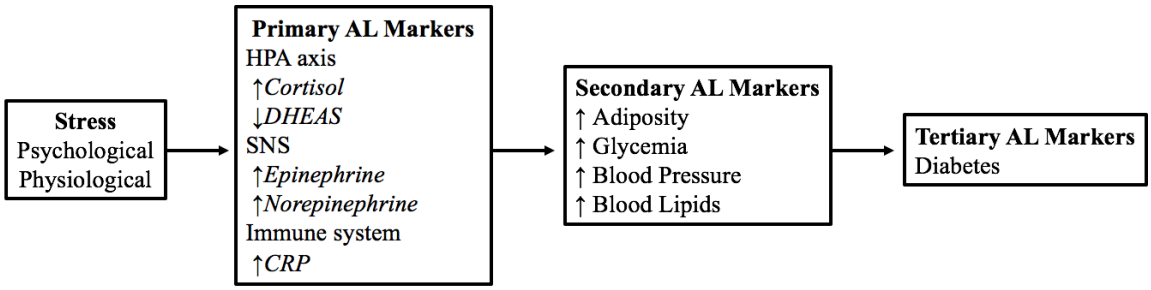


**Figure 1.1.** Conceptual model of the AL cascade of events. Model is not inclusive of all hypothesized primary, secondary and tertiary markers of AL

## Glycemic Load as a Physiological Stressor

The AL framework conceptualizes an unhealthy diet as a physiological stressor on the body.[28] Consumption of refined carbohydrates is a contributing factor to the rise in diabetes prevalence.[29–33] Glycemic load (GL) measures carbohydrate quality and quantity.[34] Studies have documented associations between GL and higher HbA1c (a secondary AL marker),[35] and higher diabetes risk (a tertiary AL marker).[36,37] However, some studies have documented that the latter association is present only in women and not in men.[38] It may be possible that GL partially contributes to diabetes development by acting as a physiological stressor that increases the demand of biological regulatory systems leading to initiation of AL. Few studies have examined this, but emerging evidence makes this hypothesis worth studying. Cross-sectional studies have shown associations between foods high in GL and some of the primary AL markers (i.e., cortisol, DHEA-S and CRP).[39–42] Additionally, an intervention study that provided

carbohydrate rich meals observed increased levels of norepinephrine (a primary AL marker) following these meals.[43] However, several research gaps exist. For example, the longitudinal relationship between refined carbohydrates and primary AL markers has not been evaluated, thus there is a need for longitudinal studies to better understand associations between refined carbohydrates and primary AL markers. In addition, very few studies have used a comprehensive set of biomarkers that represent dysregulation of the HPA axis, SNS and immune system, thus there is also a need for studies that include a comprehensive set of biomarkers to capture the primary AL stage. Lastly, given that studies have documented differences by sex in the association between GL and diabetes,[38] studies that evaluate the association between GL and primary AL markers by sex are also needed.

Research evidence of GL being associated with diabetes risk and with primary AL markers is particularly important for Puerto Ricans given that the Puerto Rican diet is characterized by foods high in GL (i.e., white rice, starchy vegetables and sugar sweetened beverages). Thus, GL may be playing a role on the initiation of AL in Puerto Ricans and may contribute to the metabolic disparities and high diabetes prevalence in Puerto Ricans, but studies are needed to test this hypothesis.

## Perceived Stress and Glucose Metabolism

Studies evaluating perceived stress and physiological dysregulation have shown results that support the AL model. For example, studies have documented positive associations between perceived stress and some of the primary AL markers (i.e., cortisol, epinephrine, norepinephrine),[39,44–46] higher HbA1c (a secondary AL markers),[47,48] and

with greater diabetes risk (a tertiary AL marker).[49,50] However, studies have documented differences by sex in the relationship between perceived stress and diabetes risk, where an association has been found in women and not in men.[51] Findings of perceived stress being associated with primary and secondary AL markers, and with diabetes risk, are of great relevance to the Puerto Rican population in the mainland U.S. given that this group experiences a great burden of social stressors (i.e., discrimination, violence, racism, poverty, acculturation and language barriers),[52,53] that may increase their overall stress levels. Therefore, perceived stress may contribute to metabolic disparities and the high prevalence of diabetes observed in Puerto Ricans.

## Puerto Ricans Experience Concurrent Psychological and Physiological Stressors

Puerto Ricans in the mainland U.S. are likely to experience concurrent stressors given the great number of social stressors that they face,[52,53] and because their traditional diet is high in GL. Thus, psychological and physiological stressors may often co-occur in this population. A study conducted in Puerto Ricans residing in the mainland U.S. documented positive associations between perceived stress and consumption of sweets (high in GL),[39] suggesting that perceived stress and high GL are concurrent in this population. The presence of concurrent psychological and physiological stressors may have a compound effect on secondary AL markers, such as glucose dysregulation (i.e., higher HbA1c), potentially contributing to a greater metabolic disadvantage in Puerto Ricans experiencing concurrent stressors compared to those who do not. However, studies have not evaluated this hypothesis. Additionally, given that both GL and

perceived stress are hypothesized to initiate AL, it is possible that, if there is an existing compound effect on HbA1c, it is partially exerted through primary AL markers. Nonetheless, studies have not examined this. Lastly, given the documented sex differences in associations between GL and perceived stress on diabetes risk,[38,51] it is important to evaluate these relationships by sex. Identifying and understanding how GL and perceived stress affect glucose metabolism and diabetes in Puerto Rican men and women, is critical to the development of novel interventions that ameliorate Puerto Rican health disparities.

## Weight Change Affects Glucose Metabolism and Stress May Moderate this Association

As previously mentioned, AL is a dynamic network, with complex causal associations occurring among biomarkers between and within the same AL stages (primary markers vs. secondary markers, vs. tertiary marker).[15,54] In particular, weight change (secondary AL marker) can affect metabolic dysregulation of glucose metabolism (secondary AL marker) and diabetes risk (tertiary AL marker).[55] For example, research studies have shown that weight gain is associated with deterioration of glucose metabolism and higher diabetes incidence.[56,57] A population-based cohort in Sweden reported that a 1 $kg/m^2$ increment during a period of 10 years, increased the risk of diabetes by 52%.[57] On the other hand, intervention studies provide evidence that weight loss leads to an improvement of glucose metabolism and decreased diabetes risk.[58–62] In the Diabetes Prevention Program (DPP), a weight loss of 15.4 lbs reduced diabetes incidence by 58% and improved HbA1c by -0.09. However, differences by sex on the

effect of weight loss have been documented, with some studies like the DPP and the Finish Diabetes Prevention Study finding that weight loss resulted in a greater reduction of glucose markers and diabetes risk in men than in women.[63,64]

Additionally, differences on the effect size of the association between weight change and parameters of glucose metabolism (i.e., HbA1c, diabetes risk) have been documented within and between studies. The Nurses' Health Study found that a increase in weight of 5kg over 20 years was associated with a 44% increase in the risk of diabetes for Latina women compared to 37% for non-Latina white women,[56] and that an increment of 5kg/$m^2$ over 20 years yielded a diabetes risk ratio of 2.21 for Latina women and 1.96 for non-Latino white women. In the Lawrence Latino Diabetes Prevention Program (LLDPP), which was conducted in Latinos of low socio-economic status (SES), a modest weight loss of 2.5lbs ($1/6^{th}$ of the weight loss achieved in the DPP) led to equivalent reductions in HbA1c (-0.1%) as in the DPP.[60] No study has examined potential factors responsible for modifying the effect of weight change on glucose metabolism.

We hypothesize that populations with greater stress may experience greater glucose sensitivity to weight change. For example, the ethnic differences documented in the effect size of the Nurse's Health Study may be due, in part, to the numerous chronic stressors that Latinos often face (i.e., acculturation, language barriers and discrimination).[52,53] The LLDPP was conducted with a low SES sample compared to the DPP (40% with educational level $\geq$ high school in the LLDPP vs. 75% with educational level $\geq$ high school in the DPP). With low SES known to be a lifelong stressor,[65,66] it may be possible that the increased HbA1c sensitivity to weight change seen in the LLDPP was

due to stress. However, there is a need for studies that test this hypothesis. Additionally, given the documented sex differences in the associations between stress and metabolic dysregulation[45,49] and between weight loss and diabetes risk,[63,64] studies that explore the interplay between weight change and stress on HbA1c by sex are needed. This may help understand how psychological stress may affect complex associations occurring among biomarkers within AL stages. It may also help identify a particular subset of individuals at greater diabetes risk, who may benefit the most from weight loss interventions.

## Specific Aims

Using data from the Boston Puerto Rican Health Study, the overarching goal of this dissertation is to examine the association between physiological and psychological stressors and glucose metabolism in Puerto Rican men and women. This dissertation sought to evaluate three Specific Aims using an adapted AL model as the conceptual framework (Figure 2):

1. **To examine the relationship between GL and a composite score of primary AL markers in Puerto Rican men and women.**
2. **To evaluate the interaction between perceived stress and GL on HbA1c in Puerto Rican men and women, and to explore if a composite score of primary AL markers mediates this interaction.**
3. **To confirm the association between weight change and change in HbA1c in Puerto Rican men and women, and test the hypothesis that there is an interaction between change in weight and perceived stress on HbA1c change.**

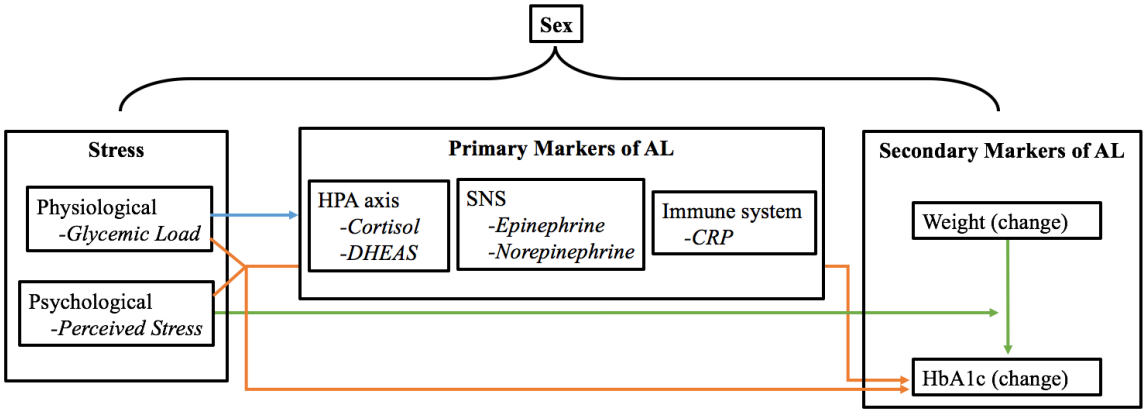


**Figure 1.2.** Conceptual model of dissertation guided by the AL framework. Blue arrow represents Aim 1, orange arrows represent Aim 2, and green arrows represent Aim 3.

# CHAPTER II:
# GLYCEMIC LOAD IS ASSOCIATED WITH PRIMARY MARKERS OF ALLOSTATIC LOAD IN PUERTO RICAN WOMEN


## ABSTRACT

**BACKGROUND:** Puerto Ricans have high prevalence of type 2 diabetes (diabetes). Dietary glycemic load (GL**)** and allostatic load (AL) are linked with diabetes. AL starts with dysregulation of primary AL markers (representing dysregulation of the hypothalamic-pituitary adrenal axis, sympathetic nervous system and immune system). GL may act as a physiological stressor, contributing to dysregulation of primary AL markers.

**OBJECTIVE:** To examine the relationship between GL and a composite score of primary AL markers.

**METHODS:** Data were from the Boston Puerto Rican Health Study, a cohort study of Puerto Ricans adults, including 262 men and 697 women with complete data at baseline and 2-year follow-up. GL was calculated from a food frequency questionnaire. Sex-specific composite score of primary AL markers included: cortisol, dehydroepiandrosterone, epinephrine, norepinephrine and C-reactive protein. Linear regression models, stratified by sex, were adjusted for baseline age, education, smoking, physical activity, BMI, use of medications for diabetes, hypertension and hyperlipidemia and menopause status (for women).

**RESULTS:** Mean baseline GL score was 155±28 for men and 135±34 for women. Mean primary AL score was 1.25±1.14 for men and 1.25±1.06 for women. GL was not associated with primary AL score in men. In women, GL change from baseline to 2 years

was positively associated with change in primary AL score, after adjusting for demographics, behavioral and biological factors, and baseline AL score ($\beta$=0.029; p=0.049), but further adjustment for medications decreased statistical significance ($\beta$=0.028; p=0.058).

**CONCLUSION:** Increasing GL over 2 years was positively associated with small increases in a composite score of primary AL markers in Puerto Rican women. GL may be an important target for interventions to reduce diabetes disparities in this population.

## INTRODUCTION

Puerto Ricans living on the mainland U.S. experience disproportionately higher prevalence of type 2 diabetes (referred to as diabetes herein) compared to other Latino sub-groups and non-Latino whites.[6] One in 6 Puerto Rican men and 1 in 5 Puerto Rican women have diabetes,[6] and diabetes prevalence has been reported to be as high as 39% among older Puerto Rican adults.[67] However, few studies have examined contributing factors to these diabetes disparities in this population.

Glycemic load (GL), a measure of low carbohydrate quality (i.e., refined carbohydrates) and quantity,[34] has been linked to increased risk of diabetes.[36,68–70] However, one meta-analysis reported sex differences, with GL associated with higher diabetes risk among women, but not men.[38] Given that the Puerto Rican diet is characterized by foods high in GL, understanding the association between GL and diabetes risk factors is particularly relevant to Puerto Ricans.

The allostatic load model (AL) posits that unhealthy dietary intake acts as a physiological stressor on the body.[28] AL is defined as the wear and tear of the body's regulatory systems due to chronic exposure to stress,[15,16] leading to chronic health conditions such as diabetes.[15,16] The concept of AL describes a cause-effect chain triggered by chronic or constant exposure to stress, which results in chronic or constant activation of the hypothalamic-pituitary adrenal axis ((HPA), represented by cortisol and dehydroepiandrosterone (DHEAS)), the sympathetic nervous system (SNS; represented by epinephrine and norepinephrine), and the immune system (represented by C-Reactive Protein (CRP)).[15,16] Chronic dysregulation of these biomarkers, called primary AL markers, leads to dysregulation of secondary AL markers, which include an increase in: adiposity, blood pressure, blood lipids and blood glucose.[15,16] In turn, the accumulation of secondary stress markers leads to chronic diseases (tertiary AL markers). Although AL is conceptualized as a cascade of events, it is often studied as a composite measure, combining primary, secondary and tertiary markers into a single score. However, the sequence of responses and their contribution to disease development,[21–24] as well as the role of sex in this biological process, have not been well studied.

Few studies have examined the hypothesis that GL acts as a physiological stressor. Previous studies, most cross-sectional, [39–42] found that a sweets dietary pattern (high in GL) was positively associated with urinary cortisol;[39] a dietary pattern high in French fries (high in GL) was negatively associated with DHEA-S;[40] carbohydrate rich meals were associated with higher post-prandial norepinephrine;[43] and GL was positively associated with CRP.[41,42] However, the few longitudinal studies to date on GL and CRP

have shown mixed results.[71,72] Methodological differences in study design and measurement of carbohydrate preclude firm conclusions. Refined carbohydrate intake may play a role on the initiation of AL in Puerto Ricans because of the cultural preference for foods high in GL, thus contributing to the metabolic disparities observed in Puerto Ricans.

**METHODS**

The current analysis used data from the Boston Puerto Rican Health Study (BPRHS), described elsewhere.[67] Briefly, between 2004-2009 the BPRHS enrolled Puerto Rican men and women, aged 45 to 75 y and residing in the Greater Boston area, using primarily door to door enumeration (in census blocks with at least 25 Hispanic adults), but also community events, referrals from recruited individuals, and flyers distributed in the community. Individuals were eligible if they self-identified as Puerto Rican, lived in the Boston metropolitan area, did not have severe cognitive impairment (Mini Mental State Examination score < 10) and planned to stay in the area for at least 2 years. A total of 2170 individuals were identified, of which 1780 were eligible to participate. Informed consent was obtained prior to conducting baseline interviews. Trained bilingual research staff conducted study interviews. This study was approved by the Institutional Review Boards of Tufts University and Northeastern University.

The BPRHS collected socio-demographic, behavioral, dietary (through an adapted food frequency questionnaire), anthropometric (measured during interviews) and biochemical (12hr urine and fasting blood samples) measures at baseline and 2 years after

baseline. The current analysis includes data obtained from men and women at both time-points. A total of 1,500 individuals (n=1,056 women; 70.4% women) completed baseline measures. Of these, 81% (n=1,221 men and women) completed the 2 y follow-up assessment. For the present analysis, individuals were ineligible if they had implausible dietary intake at either time point (n=23 for $\leq$600 kcal/day; n=56 for $\geq$4800 kcal/day). We further excluded participants with missing data for any of the primary markers of AL at baseline or 2 y follow-up (n=166) or on confounders (n=17). The final sample included 262 men and 697 women.

**MEASURES**

*Glycemic Load (GL).* GL was calculated from dietary intake, measured with a food frequency questionnaire adapted for Puerto Ricans.[73] Using the previous year as the reference period, this questionnaire includes staple foods and portion sizes that were adjusted to the Puerto Rican diet to accurately measure dietary intake. Nutrient intakes were calculated using the Nutrition Data System for Research (NDS-R) software (version 2007, Minneapolis, MN). GL was calculated, as in previous studies.[74] Briefly, the International Tables of Glycemic Index (GI) and GL values with glucose as the reference value were used. Foods that had 5g or more of total carbohydrate/medium portion size were assigned a GI value, while those with 5g or less of total carbohydrate/medium portion size were assigned a GI of zero. In order to select the most appropriate GI value, data on food preparation collected in the FFQ was used. If a specific food had more than one GI value, we used the mean value of all available GIs. For foods with different published GI values, the value from the most similar food was used. To calculate GL of a

food, the GI was multiplied by grams of available carbohydrate in one serving of the food. Lastly, the total dietary GL was calculated by summing the GL scores of all food sources. The total GL values were adjusted for energy intake using the residual method,[75] separately for men and women. For the baseline analysis, GL was used as a continuous variable. We further calculated the difference in GL values between time points (GL at year 2 – GL at baseline; positive values indicate an increase in GL and negative values a decrease) to evaluate change in GL and change in primary markers of AL. The difference calculated was also used as a continuous variable in the analysis.

*Primary AL markers.* A composite score of the primary markers of AL was used as the dependent variable. Biomarkers measured included cortisol, epinephrine, norepinephrine (each from 12hr urine), DHEA-S and CRP (both from fasting blood), representing the hypothalamic pituitary adrenal (HPA) axis (cortisol and DHEA-S), the sympathetic nervous system (SNS) (epinephrine and norepinephrine), and inflammation (CRP).[15,16] Because there are no clinical cut-off scores for most of these measures, and given sex differences in AL,[76] population and sex-specific quartiles defined by baseline values of each biomarker were created as in our previous work.[77] For each biomarker, an individual received a score of 0 if they were below the sex-specific 75$^{th}$ percentile or a score of 1 if they had values at or above the sex-specific 75$^{th}$ percentile. This was the opposite for DHEA-S. DHEA-S is an HPA axis antagonist, with lower levels representing dysregulation. Thus, values at or below the sex-specific 25$^{th}$ percentile were assigned a score of 1 and those above the sex-specific 25$^{th}$ percentile a score of 0. A sex-specific summary score for primary AL markers was then created by summing the scores

for each biomarker. The summary score ranged from 0-5. AL was used as a continuous variable for the baseline analysis. We then calculated the difference in values of primary AL markers between time points (primary AL markers at year 2 – primary AL markers at baseline; with positive values indicating an increase in primary AL markers and negative values a decrease) to evaluate change in primary AL markers. The difference calculated was also used as a continuous variable in the analysis.

*Covariates.* Covariates were determined a priori from the literature and included age, education, smoking, physical activity, medication use, menopause status (for women) and BMI, all assessed at baseline interviews. Education was measured with the question "What is the highest grade you completed in school?", and categorized as "< high school" and "≥high school graduate". Smoking was categorized as current, former or never smoker. A modified version of the Paffenbarger questionnaire was used to measure physical activity; the calculated score was used as a continuous variable. Women reported their menopause status by answering the question "Have you already gone through or are you currently going through menopause?" (yes/no). Medications for diabetes, hypertension and hyperlipidemia were self-reported and, if available, interviewers recorded information from labels/bottles. Lastly, BMI ($kg/m^2$) was calculated from height and weight measures taken by trained study staff.

**ANALYSIS**

All analyses were, a priori, stratified by sex. Descriptive statistics at baseline include frequencies for categorical variables and mean and standard deviation for continuous variables. Multivariable linear regression analyses by sex were used to

evaluate the association between GL and the composite score of primary AL markers at baseline, and to evaluate the association between change in GL and change in the composite score of primary AL markers between baseline and 2 years (both unadjusted and adjusted for baseline composite score of primary markers of AL). For each analysis we conducted an unadjusted model (model 1) and a series of adjusted models: model 2 = age and BMI (and menopause status for model in women); model 3 = model 2 + education + smoking + physical activity; and model 4 = model 3 + use of medications. Significance was set at $p<0.05$. STATA version 14 was used for all analyses.

**RESULTS**

The sample was 70% women, had a mean age of 57±8 years, and more than 60% had < high school education (Table 1). Most were overweight or obese, with sedentary or lightly active lifestyle. The majority of women were in menopause. Half of the women and one third of men were never smokers. Approximately one-third of the sample were taking medications for diabetes, one half for hypertension and 40% for dyslipidemia. The mean GL score at baseline was 155±28 for men and 135±34 for women and it decreased an average of 16 points for men and 18 points for women from baseline to year 2. The mean composite score of primary AL markers was 1.25 for both men and women, and it increased to 1.46 among men and 1.48 among women by year 2.

GL was not significantly associated with the composite score of primary AL markers at baseline (Table 2). In men, change in GL from baseline to 2 y was not associated with change in the composite score of primary AL markers. In women, greater change in GL scores from baseline to 2 y was associated with small increases in the

composite score of primary AL markers in models adjusted for age, BMI, menopause status, behavioral factors and baseline primary AL markers. This association became marginally significant in models further adjusted for use of medications (model 4), but coefficients remained similar. Results were also similar in models that did not adjust for baseline composite score of primary AL markers.

**DISCUSSION**

To our knowledge, this is the first study to evaluate the association between GL and a composite score of primary stress markers of AL. We found that neither GL at baseline or change in GL were significantly associated with the composite score of primary stress markers of AL in men. However, findings in women showed that greater change in GL from baseline to 2 years was associated with small increases in the composite score of primary AL markers, which confirms the hypothesis of GL being a physiological stressor and contributing to dysregulation of primary AL markers. The latter finding is consistent with associations between other measures of carbohydrate consumption and individual primary AL markers observed in several cross-sectional studies. An analysis with baseline BPRHS data (including both men and women) previously showed that a sweets dietary pattern (defined by foods high in GL) was positively associated with urinary cortisol.[39] Another baseline BPRHS analysis, that included both men and women, showed that a dietary pattern characterized by high intake of French fries (high in GL) was negatively associated with the HPA axis antagonist DHEA-S.[40] In addition, two cross-sectional studies using data from the Women's Health Study showed positive associations between GL and CRP.[41,42] Lastly, an experimental

study that supplied a carbohydrate rich meal showed an increase in norepinephrine following the carbohydrate rich meal.[43] Thus, our findings of an association between increases in GL and increases in a composite score of primary AL markers among women are in line with these studies and provide additional evidence given that we evaluated changes in GL and changes in primary AL markers.

Few other studies have evaluated the association between GL and a composite score of AL (including primary and secondary markers). One study, conducted with Japanese women, found that intake of vegetables (a food group low in GL) was associated with low AL.[78] In addition, cross-sectional data from BPRHS men and women showed that a dietary pattern characterized by intake of French fries (a food high in GL), was associated with higher AL.[40] Thus, although these studies do not directly evaluate GL and included other secondary markers of AL, they provide indirect support for our findings.

The majority of the available studies are cross-sectional and provide some evidence of GL being associated with primary markers of AL.[39–42,78] However, our cross-sectional analysis with baseline data did not show that GL was significantly associated with the composite score of primary AL markers. This discrepancy may be due to the different ways in which intake of refined carbohydrate is measured (GL vs. dietary patterns vs. specific foods high in carbohydrates) or to the different outcomes evaluated (composite score of only primary AL markers vs. each primary AL marker individually vs. composite score of all AL markers). Additionally, the lack of association between GL and the composite score of primary AL markers at baseline may be due to the fact that

many of those with diabetes, who are also likely to have higher primary markers of AL,[79,80] had already made dietary changes (due to their diabetes diagnosis) to decrease sugar intake and therefore GL. It is also possible that, biologically, the exposure to an increase in GL over time is what influences these primary markers, which is consistent with the AL model. Due to this discrepancy and the lack of longitudinal analyses in the literature evaluating GL and primary AL markers, longitudinal studies are needed to truly understand this relationship.

In our sample, GL was not associated with the composite score of primary AL markers in men. Our sample size for men was smaller than that for women, which may account, in part, for non-significant results. It is also possible that GL is not associated with primary AL markers in men. As previously mentioned, sex differences in the association between GL and risk of diabetes have been reported,[38] where, consistent with our findings, it was only significant for women.

Our findings fill important research gaps in that they are based on longitudinal data and a comprehensive set of biomarkers to capture the primary AL phase. Most of the previous studies examining associations between refined carbohydrates and primary AL markers examined individual markers and use a cross-sectional design.[39–42,78] With data available for two time points, our study was able to consider change in GL over 2 y with change in primary markers of AL over the same time period. Previous studies do not fully evaluate the primary AL response, but evaluate primary AL markers individually,[39–42] or incorporate the primary markers along with the secondary AL markers.[40,78] Our study

incorporated a composite score of primary AL markers that represent the systems that are first activated in the stress response: HPA axis, SNS and the immune system.

Overall, our findings that an increase in GL is associated with an increase in a composite score of primary markers of AL suggest that GL may be a physiological stressor for women that contributes to dysregulation of primary AL markers. Animal studies provide evidence that carbohydrates may stimulate the SNS and, thus, increase release of some primary AL markers (i.e., epinephrine and norepinephrine).[81,82] Because some of these markers are known to increase blood glucose concentration,[83,84] and are hypothesized to trigger dysregulation of secondary markers of AL,[15,28] which include hyperglycemia, understanding the relationship between GL and primary AL markers may help in understanding how high GL influences glucose metabolism and diabetes. This is of great importance to Puerto Ricans, given their cultural preference for foods high in GL (i.e., white rice, sugary drinks and starchy vegetables) and their high prevalence of diabetes. However, more longitudinal studies are needed to confirm our findings an to understand the observed sex differences. In addition, intervention studies that aim to improve and lower GL scores are also needed to test if reduction of GL ameliorates primary AL markers.

The study results should be considered with certain limitations and strengths in mind. One limitation is that GL was measured with a FFQ and calculated from self-reported data that is susceptible to bias. However, the FFQ used in this study was specifically adapted for this population by including ethnically appropriate foods and recipes and has been validated against 24-hour dietary recalls in Latinos.[73] It is important

to mention that a portion of participants were excluded due to missing data (16% in women and 15% in men). However, missingness was mainly due to primary markers of AL (90% of missing cases in women and 94% in men). Excluded women due to missing data were similar to included women in all covariates, but they had slightly lower baseline GL and subsequently lower changes in GL (8-unit difference). Similarly, excluded men due to missing data were similar to included men in all covariates and in baseline GL, but they had greater changes in GL (25-unit difference). Another limitation is that the study included only Puerto Ricans, which may limit generalizability of its findings to other Latino groups. However, the focus on Puerto Ricans is also a strength, as Puerto Ricans comprise the largest Latino group in the North East of the U.S.,[85] and experience considerable disparities in diabetes,[86] but have been underrepresented in research. In addition, a strength of our analysis is the use of a composite measure of primary AL markers that captures overall dysregulation of the HPA axis, SNS and the immune system, and the availability of longitudinal data that allowed us to explore changes in GL and changes in primary AL markers.

In conclusion, an increase in GL over 2 years was associated with a small increase in a composite score of primary AL markers in women. Studies with larger samples of men are needed to understand this relationship in men. In addition, more longitudinal studies are needed to understand the relationship between GL and dysregulation primary AL markers and to test interventions that improve GL in Puerto Rican women.

**Table 2.1.** Baseline characteristics of participants in the Boston Puerto Rican Health Study.

| | **Men n=262 (27.3%)** | **Women n=697 (72.7%)** |
|---|---|---|
| ***Demographics*** | | |
| Age, mean (SD) | 56.8 (8.2) | 57.2 (7.4) |
| <High School, n (%) | 166 (63.4) | 455 (65.3) |
| ***Weight status***, mean (SD) | | |
| BMI | 29.8 (5.0) | 32.9 (6.9) |
| Experiencing Menopause | - | 576 (82.6) |
| ***Behavioral factors*** | | |
| Smoker, n (%) | | |
| Never | 80 (30.5) | 359 (51.5) |
| Former | 97 (37.0) | 190 (27.3) |
| Current | 85 (32.4) | 148 (21.2) |
| Physical activity score, mean (SD) | 32.7 (5.9) | 31.1 (4.0) |
| ***Medication Use,* n (%)** | | |
| Medications for diabetes | 83 (31.7) | 214 (30.7) |
| Medications for hypertension | 140 (53.4) | 379 (54.4) |
| Medications for hyperlipidemia | 112 (42.8) | 289 (41.5) |
| ***GL,*** mean (SD) | | |
| Baseline | 155.7 (27.7) | 134.8 (24.2) |
| Change | -16.1 (30.1) | -17.6 (26.1) |
| ***Primary markers of AL,*** mean (SD) | | |
| Baseline | 1.25 (1.14) | 1.25 (1.06) |
| Change | 0.21 (1.27) | 0.23 (1.20) |

**Table 2.2.** Baseline and longitudinal association between GL and primary AL markers among men in the Boston Puerto Rican Health Study.

| | **Model 1** | | **Model 2** | | **Model 3** | | **Model 4** | |
|---|---|---|---|---|---|---|---|---|
| | $\beta$ (95%CI) | P value | $\beta$ (95%CI) | P value | $\beta$ (95%CI) | P value | $\beta$ (95%CI) | P value |
| **Outcome: Baseline primary markers of AL** | | | | | | | | |
| Baseline Glycemic Load* | -0.009 (-0.059, 0.041) | 0.714 | -0.006 (-0.056, 0.044) | 0.809 | -0.006 (-0.065, 0.044) | 0.825 | -0.006 (-0.055, 0.041) | 0.827 |
| **Outcome: Change in primary markers of AL** | | | | | | | | |
| Glycemic Load change* | -0.05 (-0.056, 0.047) | 0.851 | -0.007 (-0.058, 0.045) | 0.801 | -0.009 (-0.061, 0.043) | 0.722 | -0.010 (-0.062, 0.042) | 0.708 |
| Glycemic Load change adjusting for baseline AL* | 0.001 (-0.041, 0.044) | 0.957 | 0.002 (-0.041, 0.045) | 0.937 | -0.002 (-0.044, 0.041) | 0.936 | -0.002 (-0.045, 0.041) | 0.916 |

*Glycemic load and glycemic load difference are shown in increments of 10 units. Model 1= unadjusted. Model 2 = age and BMI. Model 3 = model 2 + education + smoking + physical activity. Model 4 = model 3 + use of medications

**Table 2.3.** Baseline and longitudinal association between GL and primary AL markers among women in the Boston Puerto Rican Health Study.

| | **Model 1** | | **Model 2** | | **Model 3** | | **Model 4** | |
|---|---|---|---|---|---|---|---|---|
| | $\beta$ (95%CI) | P value | $\beta$ (95%CI) | P value | $\beta$ (95%CI) | P value | $\beta$ (95%CI) | P value |
| **Outcome: Baseline Primary markers of AL** | | | | | | | | |
| Baseline Glycemic Load* | -0.014 (-0.046, 0.019) | 0.413 | -0.014 (-0.046, 0.019) | 0.407 | -0.012 (-0.044, 0.020) | 0.460 | -0.012 (-0.044, 0.019) | 0.443 |
| **Outcome: Change in Primary markers of AL** | | | | | | | | |
| Glycemic Load change* | 0.034 (0.0001, 0.069) | 0.049 | 0.034 (0.0001, 0.069) | 0.049 | 0.033 (-0.0009, 0.067) | 0.056 | 0.033 (-0.0001, 0.067) | 0.062 |
| Glycemic Load change adjusting for baseline AL* | 0.032 (0.003, 0.061) | 0.029 | 0.029 (0.0007, 0.058) | 0.045 | 0.029 (0.0001, 0.057) | 0.049 | 0.028 (-0.0009, 0.056) | 0.058 |

*Glycemic load and glycemic load difference are shown in increments of 10 units. Model 1= unadjusted. Model 2 = age, BMI and menopause status. Model 3 = model 2 + education + smoking + physical activity. Model 4 = model 3 + use of medications

# CHAPTER III:
# EVALUATING THE INTERACTION BETWEEN GLYCEMIC LOAD AND PERCEIVED STRESS ON HBA1C AND EXPLORING THE MEDIATING ROLE OF PRIMARY MARKERS OF ALLOSTATIC LOAD IN PUERTO RICAN MEN AND WOMEN


## ABSTRACT

**Background**: Type 2 diabetes (diabetes) is prevalent in Puerto Ricans. Stress and glycemic load (GL) are associated with diabetes and with primary markers of allostatic load (AL). Puerto Ricans have high stress burden and high GL diets. No study has examined the interplay between stress and GL on HbA1c and whether primary AL markers mediate this interaction.

**Objectives**: To evaluate the interaction between perceived stress and GL on HbA1c in Puerto Rican men and women, and explore the mediating role of primary AL markers.

**Methods**: Baseline data from the Boston Puerto Rican Health Study included 356 men and 914 women. GL was calculated from a food frequency questionnaire. Perceived stress was measured with the 14-item Perceived Stress scale. A sex-specific composite score of primary markers of AL was used as mediator. HbA1c, obtained from fasting blood samples, was log-transformed for analyses. Analyses included multivariable linear regression models, stratified by sex, and likelihood ratio tests for interactions. Mediation was tested with the Baron and Kenny method.

**Results**: Mean age was 57 y. Mean HbA1c was 6.97%$\pm$1.89 in men and 7.11%$\pm$1.72 in women. The interaction between perceived stress and GL on HbA1c was not statistically significant in men ($p>0.05$), but it was in women ($p<0.05$). Women with high perceived stress-high GL had marginally higher log-transformed HbA1c than the high perceived

stress-low GL group ($\beta$:-0.025; p=0.090) and low perceived stress-high GL group ($\beta$:-0.026; p=0.073). Primary markers of AL did not mediate this interaction.

**Conclusions**: In Puerto Rican women, perceived stress and GL had a compound effect on HbA1c that was not mediated by primary markers of AL. More studies are needed to confirm our findings.

## INTRODUCTION

Type 2 diabetes (diabetes) is a world wide epidemic.[87] This public health problem is a greater burden in Puerto Ricans, with a prevalence of diabetes of 19%[10] and of pre-diabetes of 34%.[88] Puerto Ricans are the second largest Latino group in the mainland U.S.,[89] thus it is important to understand factors that contribute to the high prevalence of pre-diabetes and diabetes in this group.

The allostatic load (AL) model explains how stress influences disease development, including diabetes. [15,16] AL captures "the wear and tear of the body's regulatory systems in response to chronic stressors", and is conceptualized as a cascade of events.[15,16] In the presence of a chronic stressor, the hypothalamic-pituitary adrenal axis (HPA), sympathetic nervous system (SNS) and the immune system are continuously activated, represented by dysregulation of primary AL markers (cortisol, epinephrine, norepinephrine, dehydroepiandrosterone (DHEAS) and c-reactive protein (CRP)). Accumulation of dysregulated primary AL markers leads to secondary AL markers, which include dysregulation of glucose metabolism. Lastly, tertiary AL markers, or disease endpoints such as diabetes, occur.

Consistent with the AL model, studies have shown that perceived stress is associated with primary AL markers (i.e., cortisol, norepinephrine epinephrine),[39,44,45] while other studies have evidenced positive associations with secondary AL markers, such as glycosylated hemoglobin (HbA1c).[47] Moreover, previous research has linked perceived stress with greater diabetes incidence (tertiary AL marker),[49] but some studies have not been able to confirm this association in men.[51] Given the evidence linking stress with dysregulation of glucose metabolism (higher HbA1c) and diabetes risk, and the fact that Puerto Ricans encounter numerous social stressors (i.e., discrimination, racism, acculturation and language barriers),[52,53] it is important to consider stress as a contributor to the high prevalence of pre-diabetes and diabetes in this minority group.

Intake of refined carbohydrates, as measured by glycemic load (GL), is hypothesized to be associated with AL and is thought to be an important contributor to dysregulation of glucose metabolism.[35,36,38] Previous studies have shown positive associations between foods high in GL and some primary markers of AL,[39,40] and with higher HbA1c.[47,48] Other studies have shown associations between GL and greater risk of diabetes,[49,50] but this association has been mainly documented in women.[38] These findings are also of great importance to Puerto Ricans, as the traditional Puerto Rican diet is characterized by foods high in GL (i.e., white rice, starchy vegetables and sugar-sweetened beverages). Thus, GL may also be contributing to the high prevalence of pre-diabetes and diabetes in this minority group.

Because Puerto Ricans experience numerous stressors and their diet can be considered a physiological stressor (high GL), they may experience concurrent

psychological and physiological stress. In fact, a study conducted in Puerto Ricans residing in the mainland U.S. showed that perceived stress was positively associated with intake of sweets (a dietary pattern high in GL), providing evidence that these two stressors may co-occur in this population.[39] Experiencing concurrent stressors may have a compound effect on HbA1c, but studies have not tested this. It is also unknown if there is a biological mechanism (i.e., primary AL markers) behind the interaction of psychological and physiological stress on HbA1c, and, given the documented sex differences in stress and diabetes risk,[51] whether sex plays a role in this interaction. Thus, the objectives of this study were to 1) evaluate the interaction between perceived stress and GL on HbA1c in Puerto Rican men and women, and 2) explore if primary AL markers mediated this interaction, using data from the Boston Puerto Rican Health Study (BPRHS).

**METHODS**

This cross-sectional analysis used baseline data from the BPRHS.[67] The BPRHS enrolled Puerto Rican men and women, aged 45 to 75 y and residing in the Greater Boston area between the years of 2004-2009. Recruitment efforts included door to door enumeration and community outreach strategies. Eligibility criteria included: self-identify as Puerto Rican, no severe cognitive impairment and planning to stay in the Boston area for at least 2 years. A total of 1,500 individuals (70.4% women) enrolled in the study. Participants provided informed consent prior to interviews, which were conducted by bilingual study personnel. The Institutional Review Boards at Tufts University and Northeastern University approved the study.

Demographics, dietary, biological and anthropometric measures were collected during study interviews. For this analysis, men and women with implausible dietary intake (<600kcal and >4800 kcal; n= 72) and with missing data on exposure (n=0), outcome (n=29), moderator (n=17), mediators (n=80), and confounders (n=12) were excluded. In all, a total of of 356 men and 914 women were included in the analytic sample. Included and excluded participants were similar in all characteristics, except for diabetes status, with excluded individuals due to missing data being more likely to have pre-diabetes.

**MEASURES**

*Glycemic Load.* Dietary intake was measured with a food frequency questionnaire (FFQ),[73] and was used to calculate GL. This FFQ was specially adapted for this population and included staple foods from the Puerto Rican diet and adjusted portion sizes for this group.[73] The Nutrition Data System for Research (NDS-R) software (version 2007, Minneapolis, MN) was used to calculate nutrient intakes. As in previous analyses of the BPRHS,[74] GL was calculated with the International Tables of Glycemic Index (GI) and GL values using glucose as reference. The GL of a food was calculated by multiplying the GI of the food by grams of available carbohydrate in one serving of such food. We then calculated the total dietary GL of each individual by summing the GL scores of all consumed foods. We adjusted the total GL values for energy intake using the residual method.[75] This adjustment was done separately for men and women. Given that there are no clinical cutoff scores of GL, we categorized GL as low and high according to

the sex-specific GL median (median for men = 158; median for women = 133) as other studies have used population specific cut-off scores to categorize GL.[36]

*Perceived stress*. Perceived stress was measured with the widely used 14-item Perceived Stress Scale.[90] This scale is a global measure of stress that assessed "the degree to which situations in one's life are appraised as stressful". For example, using the previous month as the reference period, one questions asked: "In the last month, how often have you been upset because of something that happened unexpectedly?". Response options were: never (0), almost never (1), every now and then (2), often (3) and very often (4). The total score was calculated by adding up responses for all 14 items. Scores ranged from 0-56, with higher scores suggesting greater distress. Because there are no clinical cutoff scores for this scale, we categorized perceived stress as low or high, according to the sex-specific median (median for men = 23; median for women = 25), consistent with previous studies.[39]

*Primary AL markers.* Primary markers of AL were obtained from 12-hr urine samples and fasting blood samples, as described elsewhere.[22] We used a composite score that included biomarkers representing the HPA axis, SNS and the immune response: cortisol, epinephrine, norepinephrine (all from 12hr urine samples) and DHEA-S and CRP (both from 12-hr fasting blood samples). Due to the lack of clinical cut-off scores for these biomarkers, we used population and sex-specific quartiles for each biomarker to calculate the composite score.[77] For each biomarker, a point was assigned if the value was above the sex-specific 75$^{th}$ percentile. This was the opposite for DHEA-S given that this biomarker is an HPA axis antagonist. We then added the scores of each biomarker to

create a sex-specific summary score for primary AL markers. The composite score ranged from 0-5 and was used as a continuous variable in the analysis.

*HbA1c.* HbA1c was obtained from 12hr-fasting blood samples. HbA1c values were log-transformed because the distributions for both men and women were skewed. Log-transformed HbA1c was used as a continuous variable in all analyses.

*Covariates.* We considered the following covariates a priori for the present analysis: age, education, smoking, physical activity, diabetes status, menopause status and medications for hypertension and hyperlipidemia, all self-reported by questionnaire at the baseline visit. Education was categorized into "<high school" and "≥high school graduate" due to the low education level of our sample. A modified version of the Paffenbarger questionnaire was used to measure physical activity.[91,92] For menopause status, women were asked "Have you already gone through or are you currently going through menopause?" and they answered yes or no. Given that our outcome was HbA1c, we used a diabetes status variable that incorporated levels of blood glucose as defined by the American Diabetes Association[93] and type of medications used. Participants were categorized as non-diabetics if they were not taking any glucose lowering medications and had blood glucose levels < 100mg/dL; as pre-diabetics if they were not taking any glucose lowering medications and had blood glucose between 100-125mg/dL; as diabetics without medications if they were not taking any glucose lowering medications but had blood glucose levels > 125mg/dL; as diabetic with medications if they were taking any type of glucose lowering medication that was not insulin, regardless of blood

glucose concentration; and as diabetic with insulin if they were using insulin, regardless of blood glucose concentration and other medication use.

**ANALYSIS**

We stratified all analyses by sex. Sample characteristics were contrasted by GL status using the chi-square test for categorical variables and t-test for continuous variables. Multivariable linear regression models, stratified by sex, were used to evaluate the interaction between perceived stress and GL using log-transformed HbA1c as the outcome. A likelihood ratio test was used to examine significance of the interaction terms and, if significant, linear predictors were presented using high perceived stress and high GL as the reference group. All models were adjusted for age, education, diabetes status, medications for hyperlipidemia and hypertension, menopause status (for women only), smoking and physical activity. We further tested for the potential mediating role of primary stress markers of AL in this interaction by using the Baron and Kenny method, where the total effect (c) of the predictor is decomposed into a direct (c') and an indirect component (c - c').[94] This method consisted of 4 steps, all of them using multivariable linear regressions, adjusted for covariates. The first step evaluated the association between the interaction term (GL and perceived stress) and the outcome (log-transformed HbA1c). The second step examined the association between the interaction term (GL and perceived stress) and the mediator (composite score of primary stress markers of AL). The third step evaluated the association between the mediator (composite score of primary stress markers of AL) and the outcome (log-transformed HbA1c), taking the interaction term into account. The fourth and final step evaluated the association between

the interaction term (GL and perceived stress) and the outcome (log-transformed HbA1c), adjusting for the mediator. If mediation was present, we further evaluated the mediating effect by calculating the mediated proportion with the following equation: ((c - c')/c)*100.[95,96] Significance was set at $p<0.05$ for all analyses. STATA version 14 was used for all statistics.

**RESULTS**

Men had a mean age of 57 y, the majority had a less than high school education and only one third were never smokers (Table 1). About 40% of men had diabetes and were taking lipid lowering medications. Men were mainly sedentary and mean HbA1c was 6.97%. Men in the high GL group were less likely to be on medications for hypertension and had lower log-transformed HbA1c. Women had a mean age of 57 y, the majority had a less than high school education and half were never smokers. About 40% of women were taking lipid lowering medications, more than half were taking medications for hypertension, and most were in menopause. Most women were sedentary and had mean HbA1c of 7.11%. Women in the high GL group were more likely to be free of diabetes, and had marginally lower log-transformed HbA1c. Lastly, the proportion of individuals experiencing high perceived stress did not differ by GL group in either men and women. The mean composite score of primary markers of AL also did not differ by GL group.

There was no interaction between perceived stress and GL on HbA1c (p-value LR test=0.738) in men. Thus, results are not shown and mediation was not tested.

In women, there was a significant interaction between perceived stress and GL on HbA1c (p-value LR test=0.024) (Table 2). Log-transformed HbA1c was lower in all groups compared to the high perceived stress-high GL group, but this was only marginally significant in the high perceived stress-low GL and low perceived stress-high GL groups. When testing for mediation, we found that the second step of the mediation process was not met; the interaction term was not a significant predictor of the composite score of primary markers of AL (data not shown; $p>0.05$). In addition, when primary AL markers were added in the main effects model, the coefficients remained the same. Thus, no further mediation steps were evaluated.

**DISCUSSION**

To our knowledge, this is the first study, to evaluate the interaction between psychological stress, defined as perceived stress, and GL, a physiological stressor, on HbA1c. Our results show a significant interaction between perceived stress and GL on HbA1c in women, where women with high scores on both factors had higher log-transformed HbA1c. However, this interaction was not mediated by primary markers of AL. In addition, this interaction was not observed in men.

Our results of a significant interaction between perceived stress and GL on HbA1c in women partially confirmed our hypothesis of concurrent stressors having a compound effect on HbA1c. Although no study has specifically tested this interaction, our findings are in line with other research studies that have documented individual associations between perceived stress and HbA1c and diabetes.[47–49,97,98] For example, a study in Australian women (n=12,844) found that moderate/high perceived stress was

associated with twice the risk of developing diabetes.[49] Our results are also in agreement with studies showing positive associations between GL and HbA1c and diabetes, [35,36] where one meta-analysis documented that high GL was associated with a 10% higher risk of diabetes.[36] Thus, our study fills an important research gap in that it evaluates the compound effect of two concurrent stressors on HbA1c. Our findings help understand how these two stressors, that are a great burden in the Puerto Rican population, are associated with glucose metabolism.

Our results showed that, overall, women in the high perceived stress and high GL group had higher log-transformed HbA1c. However, this association was marginally significant in the high perceived stress-low GL and low perceived stress-high GL groups. In addition, women in the low perceived stress and low GL group did not have significantly lower log-transformed HbA1c. This may be due to the fact that women with diabetes, who have higher HbA1c, may have already modified their dietary intake to reduce foods high in sugar and thus have lower GL. Additionally, although diabetic patients have disease-specific stress,[99] it is possible that women in this group (low GL low perceived stress) may have learned to manage and cope with their condition and with diabetes related stress. Nonetheless, the $\beta$ coefficients of all comparison groups were negative and in the direction that we had expected. This suggests that there may be a subset of women, who concurrently experience psychological stress and have diets high in GL, at greater metabolic disadvantage and at higher risk of diabetes. However, these findings need to be confirmed in future studies. These research studies may help uncover

specific target populations at greater risk of disease and that may benefit the most from interventions that target GL and aim to reduce stress.

In our sample, there was no interaction between perceived stress and GL in men. This may be because our sample size for men was smaller than that for women, limiting power. It is also possible that these stressors are not associated with HbA1c in men. Other studies have also shown that the associations between GL and diabetes,[38] and between perceived stress and diabetes,[51] were only present in women and not in men. Additionally, studies have also shown that perceived stress was more strongly associated with markers of AL in women than in men,[100] consistent with our results.

Consistent with the AL model, we hypothesized that the interaction between perceived stress and GL on HbA1c was mediated by a composite score of primary markers of AL.[15,16] However, our results did not confirm this hypothesis. Previous studies have shown that, individually, perceived stress and GL are associated with primary markers of AL.[39,40,44,101] In most studies, however, the association between these stressors was evaluated with each individual primary marker of AL rather than with a composite score of these markers, which may explain differences in findings. Thus, contrary to the AL model, our results with baseline data suggest that this interaction may exert effects on HbA1c independently of primary markers of AL. However, longitudinal studies are needed to evaluate if primary markers of AL mediate the interaction between GL and perceived stress on HbA1c and to confirm the AL model.

There are several limitations of our analysis. First, GL was measured with a FFQ. FFQs are based on self-reported dietary intake, thus responses may be biased due to

social desirability. Nonetheless, our FFQ was modified and validated in this population.[73] Another limitation is the cross-sectional design of our analysis, which may preclude conclusions about causality. However, to our knowledge, this is the first study that evaluates the interaction between psychological stress and GL. Lastly, our study was conducted in a sample of Puerto Ricans residing in the Northeast of the U.S. Thus, it is unknown if our findings can be generalizable to other Latino groups or to Puerto Ricans residing elsewhere. Nevertheless, this is study strength given that Puerto Ricans have the highest prevalence of diabetes among Latinos and are the second largest Latino group in the U.S.[89]

In conclusion, our results show that there was a significant interaction between perceived stress and GL on HbA1c in Puerto Rican women, but not in Puerto Rican men. It also showed that this interaction was not mediated by a composite score of primary markers of AL. Longitudinal studies are needed to confirm our findings, to evaluate if primary markers of AL mediate this interaction over time, and to test the AL model. If our findings are confirmed, research on diabetes prevention efforts in women should target reduction of stress and GL.

**Table 3.1.** Sample characteristics by GL among men in the Boston Puerto Rican Health Study.

| | **Total Sample n=356 (%)** | **Low GL n=178 (%)** | **High GL n=178 (%)** | **p value** |
|---|---|---|---|---|
| Age; mean (SD) | 56.9 (8.1) | 56.9 (7.7) | 57.1 (8.5) | 0.535 |
| < High school | 227 (63.8) | 115 (64.6) | 112 (62.9) | 0.741 |
| Diabetes status | | | | 0.447 |
| No diabetes | 131 (36.8) | 60 (33.7) | 71 (39.9) | |
| Pre-diabetes no medication | 81 (22.8) | 38 (21.4) | 43 (24.2) | |
| Diabetes without medications | 25 (7.0) | 13 (7.3) | 12 (6.7) | |
| Diabetes with oral medications | 78 (21.9) | 42 (23.6) | 36 (20.2) | |
| Diabetes with insulin | 41 (11.5) | 25 (14.0) | 16 (9.0) | |
| Lipid-lowering medication | 145 (40.7) | 72 (40.5) | 73 (41.0) | 0.914 |
| Hypertension medication | 184 (51.7) | 102 (57.3) | 82 (46.1) | 0.034 |
| Smoking | | | | 0.264 |
| Never | 109 (30.6) | 61 (34.2) | 48 (27.0) | |
| Former | 130 (36.5) | 59 (33.2) | 71 (39.9) | |
| Current | 117 (32.9) | 58 (32.6) | 59 (33.1) | |
| Physical activity score; mean (SD) | 32.5 (6.0) | 32.9 (6.3) | 32.1 (5.6) | 0.198 |
| High PSS | 161 (45.2) | 80 (44.9) | 81 (45.5) | 0.915 |
| Primary markers of AL; mean (SD) | 1.26 (1.14) | 1.22 (1.14) | 1.30 (1.13) | 0.515 |
| *Outcome* | | | | |
| HbA1c (%); mean(SD) | 6.97 (1.89) | 7.13 (1.82) | 6.80 (1.95) | 0.105 |
| Log HbA1c (%); mean(SD) | 1.91 (0.23) | 1.94 (0.23) | 1.89 (0.24) | 0.042 |

GL=glycemic load; PSS=perceived stress scale. Median GL for men = 158. Median PSS score for men = 23.

**Table 3.2.** Sample characteristics by GL among women in the Boston Puerto Rican Health Study.

| | **Total Sample n=914 (%)** | **Low GL n=457 (%)** | **High GL n=457 (%)** | **p value** |
|---|---|---|---|---|
| Age; mean (SD) | 57.4 (7.4) | 56.9 (7.4) | 57.9 (7.4) | 0.067 |
| < High school | 600 (65.6) | 289 (63.2) | 311 (68.1) | 0.125 |
| Diabetes status | | | | 0.018 |
| No diabetes | 356 (39.0) | 164 (35.9) | 192 (42.0) | |
| Pre-diabetes no medication | 200 (21.8) | 96 (21.0) | 104 (22.8) | |
| Diabetes without medications | 61 (6.7) | 29 (6.4) | 32 (7.0) | |
| Diabetes with oral medications | 207 (22.7) | 109 (23.8) | 98 (21.4) | |
| Diabetes with insulin | 90 (9.8) | 59 (12.9) | 31 (6.8) | |
| Lipid-lowering medication | 383 (41.9) | 190 (41.6) | 193 (42.2) | 0.841 |
| Hypertension medication | 503 (55.0) | 254 (55.6) | 249 (54.5) | 0.740 |
| Menopause | 763 (83.5) | 374 (81.8) | 389 (85.1) | 0.182 |
| Smoking | | | | 0.457 |
| Never | 472 (51.6) | 230 (50.3) | 242 (53.0) | |
| Former | 257 (28.1) | 137 (30.0) | 120 (26.3) | |
| Current | 185 (20.2) | 90 (19.7) | 95 (20.7) | |
| Physical activity score; mean (SD) | 31.1 (4.0) | 31.0 (3.7) | 31.1 (4.3) | 0.778 |
| High PSS | 445 (48.7) | 220 (48.1) | 225 (49.2) | 0.741 |
| Primary markers of AL; mean (SD) | 1.26 (1.06) | 1.28 (1.09) | 1.24 (1.02) | 0.532 |
| *Outcome* | | | | |
| HbA1c (%); mean(SD) | 7.01 (1.72) | 7.11 (1.72) | 6.91 (1.71) | 0.085 |
| Log HbA1c (%); mean(SD) | 1.92 (0.21) | 1.94 (0.22) | 1.91 (0.21) | 0.057 |

GL=glycemic load; PSS=perceived stress scale. Median GL for women = 133. Median PSS for women = 25.

**Table 3.3.** Interaction between perceived stress and GL on log-transformed HbA1c among women in the Boston Puerto Rican Health Study.

| | ***β*** **(95%CI)** | **p value** |
|---|---|---|
| High PSS-High GL | Reference | |
| High PSS-Low GL | -0.025 (-0.054, 0.004) | 0.090 |
| Low PSS-High GL | -0.026 (-0.055, 0.002) | 0.073 |
| Low PSS-Low GL | -0.006 (-0.034, 0.023) | 0.699 |

GL=glycemic load; PSS=perceived stress scale. Model adjusted for: age, education, diabetes status, medication for hypertension and hyperlipidemia, smoking, physical activity and menopause. p value Likelihood Ratio test = 0.024.

# CHAPTER IV:
# CHANGES IN PERCEIVED STRESS MODERATE THE ASSOCIATION BETWEEN WEIGHT CHANGE AND HBA1C CHANGE IN PUERTO RICAN WOMEN


## ABSTRACT

**Background:** Puerto Ricans experience high burden of type 2 diabetes (diabetes), pre-diabetes and obesity. Weight change influences glucose metabolism but sex differences have been documented. Additionally, the effect size of the association between weight change and HbA1c seems to vary within and between studies, with groups experiencing chronic stressors (i.e., Latino ethnicity and low socioeconomic status) potentially being more metabolically sensitive to weight change. Stress may moderate this association, but it has not been tested.

**Objective:** To assess the association between weight change and change in HbA1c in Puerto Rican men and women, and to evaluate the moderating role of perceived stress in this association.

**Methods:** Baseline and 2-year follow-up data from the Boston Puerto Rican Health Study were used. Individuals using diabetes medications were excluded. The sample included 220 men and 552 women. Perceived stress was measured with the Perceived Stress Scale-14 and HbA1c was obtained from fasting blood samples. Changes in weight, perceived stress, and HbA1c from baseline to year 2 were calculated (i.e., year 2 – baseline). Analyses included multivariable linear regression models stratified by sex. Likelihood ratio (LR) tests were used to evaluate interactions between weight change and change in perceived stress.

**Results:** Mean baseline to year 2 change in weight was 0.3 kg ± 6.1 for women and 0.3 kg ± 4.7 for men. Mean change in HbA1c was 0.03% ± 0.87 for women and -0.07% ± 1.16 for men. Weight change was not associated with HbA1c change in men. In women, weight change was associated with HbA1c change ($\beta$=0.035; p<0.001). There was an interaction between change in weight and in perceived stress, where the effect of weight change on HbA1c change was greater with increasing changes in perceived stress. **Conclusion:** Weight change was associated with change in HbA1c in Puerto Rican women, and change in perceived stress increased HbA1c sensitivity to weight change. More longitudinal studies are needed to confirm our findings.

## INTRODUCTION

One in every five Puerto Ricans living in the mainland U.S. is estimated to have type 2 diabetes (herein referred to as diabetes) and one in every three has pre-diabetes.[10,88] Similarly, one in six individuals in Puerto Rico is estimated to have diabetes and one in every three, pre-diabetes.[102] With these alarming statistics, it is imperative to understand what factors contribute to diabetes disparities among Puerto Ricans.

Weight change is a critical factor influencing glucose metabolism.[56,103] Studies have shown that weight gain is associated with greater diabetes incidence,[56] and intervention studies have shown that weight loss reduces and diabetes risk. For example, in a European population-based cohort, an increment of 1kg/m$^2$ increased the risk of diabetes by 52%.[57] Conversely, the Diabetes Prevention Program (DPP) showed that a weight loss of 15.4 lbs reduced diabetes incidence by 58% and improved glycosylated

hemoglobin (HbA1c) by -0.09%.[58] However, a more recent analysis of the DPP showed sex differences in the effect of the intervention, where weight loss resulted in a greater reduction of glucose markers in men than in women,[63] but more research is needed to understand sex differences in this relationship.

The effect size of weight change on glycemic outcomes has also been variable within and between studies. In the Nurses' Health Study, an increase in weight of 5 kg was associated with a 44% increased risk of diabetes for Latina women and a 37% increased risk for non-Latina white women.[56] Conversely, in the Lawrence Latino Diabetes Prevention Program (LLDPP), conducted with a Latino sample of low socioeconomic status (SES), a weight loss of 2.5 lbs ($1/6^{th}$ of the weight loss achieved in the DPP) led to equivalent reductions in HbA1c as in the DPP (-0.1%).[60] Factors responsible for modifying the effect of weight change on glucose markers have not been studied, thus differences in glucose sensitivity to weight change warrant further investigation.

Stress has gained research attention for its role on glucose metabolism.[104] Studies have shown positive associations between perceived stress and HbA1c[47] and risk of diabetes.[49] Thus, it is possible that populations with greater stress may experience greater glucose sensitivity to weight change. Ethnic differences in the effect size of the Nurse's Health Study may be partially due to the numerous stressors that Latinos often face (i.e., acculturation, language barriers and discrimination).[52,53] Furthermore, differences in the effect size between the DPP and the LLDPP may be due, in part, to stress. The DPP was conducted in a sample of higher SES (75% $\geq$ high school education)[58] than the LLDPP

(40% with ≥ high school education).[60] Thus, given that low SES is a lifelong stressor,[65] the increased glucose sensitivity to weight change seen in the LLDPP sample may have been due to stress.

The potential moderating role of stress in the association between weight change and glucose metabolism is particularly important for Puerto Ricans in the mainland U.S, a group exposed to many stressors.[52,53] In addition, Puerto Ricans experience one of the highest prevalences of obesity, with 40% of Puerto Rican men and 50% of Puerto Rican women in the mainland U.S. being obese.[10] Thus, the objectives of this study are to: 1) confirm the association between weight change and change in HbA1c in Puerto Rican men and women, and 2) evaluate the moderating role of perceived stress in the association between weight change and HbA1c in Puerto Rican men and women.

**METHODS**

For the present analysis, we analyzed baseline and 2-year data from the Boston Puerto Rican Health Study (BPRHS), a cohort study previously described.[67] Puerto Rican men and women, between the ages of 45-75 y, residing in the Greater Boston area between 2004 and 2009 were recruited for the BPRHS. Eligible study participants had to self-identify as Puerto Rican, live in the Boston area, have no severe cognitive impairment (Mini Mental State Examination score < 10) and plan to stay in the Boston area for at least 2 years. In all, 1,780 individuals were eligible to participate, of which 1,500 (84%) enrolled in the study. Participants provided written informed consent prior to conducting study interviews. The study was approved by the Institutional Review Boards at Tufts University and Northeastern University.

Bilingual interviewers conducted study visits and administered questionnaires that included data on socio-demographics, behavioral, perceived stress, anthropometrics and use of medications at baseline and 2 y follow-up. At both time points, participants provided fasting blood samples. The present analysis only included individuals who completed assessments at both time points (81% retention, n=1,221). We further excluded participants if they were taking any medications for diabetes (n=383) given that these medications can cause weight gain or weight loss and influence HbA1c. Participants were also excluded if they had incomplete data for weight (n=18), perceived stress (n=13), HbA1c (n=26) or confounders (n=1 for education, n=1 for smoking, n=7 women for menopause status). This resulted in a final sample size of 220 men and 552 women.

**MEASURES**

*Weight change*. Weight was measured twice at each study visit using a beam balance. For each time point, the average weight (in kilograms (kg)) was calculated. We then calculated weight change between the two time points (weight at year 2 – weight at baseline), where positive values indicate increase in weight and negative values decrease in weight). The change in weight between time points was used as a continuous variable in all analyses.

*Perceived stress change*. We used the 14-item Perceived Stress Scale to asses perceived stress.[90] Using the previous month as the reference period, the scale assessed "the degree to which situations in one's life are appraised as stressful", without reference to the stress source. Sample items include: "how often have you been upset because of

something that happened unexpectedly?" and "how often have you felt that you were unable to control the important things in life?", with response options being never (0), almost never (1), sometimes (2), fairly often (3) and very often (4). To calculate the total score for each time point, responses to all items were summed. Total perceived stress scores ranged from 0-56. As with weight change, change in perceived stress was calculated (perceived stress score at year 2 – perceived stress score at baseline), with positive values indicating an increase in perceived stress and negative values a decrease. Change in perceived stress between time points was used as a continuous variable in all analyses.

*HbA1c change*. We obtained measures of HbA1c concentration from 12hr-fasting blood samples. Change in HbA1c was calculated (HbA1c at year 2 – HbA1c at baseline), where positive values indicate an increase in HbA1c and negative values a decrease. Change in HbA1c between time points was used as a continuous variable in all analyses.

*Covariates*. Covariates, selected a priori, include: age, education, diet quality, smoking, physical activity, menopause status (for women), BMI, baseline HbA1c, and use of selected medications (i.e., antihypertensive, antihyperlipidemic, antidepressive and medications for thyroid disease), all assessed at baseline. Age, education, diet quality, smoking, physical activity, menopause status and medication use were self-reported. Because our sample had low education levels, we categorized education as "less than high school" and "high school graduate or greater". The Healthy Eating Index (HEI) 2005 was used to measure diet quality.[105] The HEI-2005 was calculated through a culturally adapted food frequency questionnaire (FFQ).[106] To assess physical activity, a

modified version of the Paffenbarger questionnaire from the Harvard Alumni Activity Survey was used.[91,92] Menopause status was self-reported by women. Height and weight measures were taken by trained study personnel at study visits and were used to calculate BMI ($kg/m^2$). Medications for hypertension, hyperlipidemia, depression and thyroid disease were self-reported and, if available, interviewers recorded information from labels/bottles.

**ANALYSIS**

We stratified all analyses by sex. Multivariable linear regression models were used to 1) confirm the association between weight change and change in HbA1c in men and women, and 2) evaluate the interaction between weight change and perceived stress change on change in HbA1c in men and women. A likelihood ratio (LR) test was used to examine interactions. If interaction was present (p value $<0.05$), we reported the slopes of change in HbA1c on weight change while holding change in perceived stress constant at different values (in intervals of 10), ranging from very low to very high. All models were adjusted for baseline age, education, diet quality, smoking, physical activity, menopause status (for women only), BMI, HbA1c and medications for hyperlipidemia, hypertension, depression and thyroid disease. Significance was set at $p<0.05$ for all analyses. STATA version 14 was used for all statistics.

**RESULTS**

Overall, 70% of the sample was female, with mean age of 56 y, and more than 60% had less than high school education (Table 1). Most were overweight or obese, with sedentary or lightly active lifestyle and a diet quality score in need of improvement.

About a third was taking medications for hyperlipidemia and 40% were taking medications for hypertension. The majority of women were in menopause. Half of the women and one third of men were never smokers, 40% of women and 20% of men were taking medications for depression, and 10% of women and 3% of men were taking medications for thyroid diseases.

The mean change in weight from baseline to year 2 was +0.3 kg $\pm$ 6.1 for women and +0.3 kg $\pm$ 4.7 for men (Table 1). The average change in perceived stress score from baseline to year 2 was -0.7 $\pm$ 9.0 for women and -0.9 $\pm$ 8.8 for men; and mean change in HbA1c was +0.03% $\pm$ 0.87 for women and -0.07% $\pm$ 1.2 for men.

In men, weight change was not significantly associated with change in HbA1c in multivariable regression models ($\beta$=-0.006; 95% CI: -0.035, 0.0023; p value=0.689). Further adjustment for diabetes medications at the 2-year time point did not alter the model ($\beta$=-0.006; 95% CI: -0.036, 0.0024; p value=0.697). Similarly, there was no interaction between change in weight and change in perceived stress on change in HbA1c (p value LR test=0.245).

In women, weight change was significantly and positively associated with change in HbA1c in multivariable regression models (Table 2). Further adjustment for diabetes medications at the 2-year time point did not alter results of the multivariable model ($\beta$=0.031; 95% CI: 0.021, 0.041; p value<0.001). In addition, there was a significant interaction between change in weight and change in perceived stress on HbA1c (p value LR test=0.006). The $\beta$ coefficient for the interaction term between weight change and change in perceived stress was positive and statistically significant ($\beta$=0.0017; 95% CI:

0.0005, 0.0029; p value=0.006). With greater change in perceived stress from baseline to 2 years, there was a greater association between weight change and HbA1c change, as indicated by the slope of change in HbA1c on weight change when perceived stress change was held constant at different combinations of values (from very low to very high) (Table 3). These results show that the effect of weight change on HbA1c change was almost 0 for those with change in perceived stress score < -5 ($p>0.05$), but positive for change in perceived stress $\geq$ -5. To illustrate this, if a woman had a weight change of +17 kg and perceived stress change of +15, she would have had a predicted change in HbA1c of +1.03%; whereas if a woman had the same weight change (+17 kg) but a greater change in perceived stress (+35), then she would have a predicted change in HbA1c of +1.61%. Conversely, a woman with a weight change of -23 kg and a change in perceived stress of +35, would have had a change in HbA1c of -2.11%, whereas a woman with the same weight change (-23 kg) but lower change in perceived stress (+5), would have had a smaller change in HbA1c (-0.95%).

**DISCUSSION**

The present study evaluated the association between weight change and HbA1c change in Puerto Rican men and women, a population with high burden of stress, obesity, pre-diabetes and diabetes. In addition, this is the first study, to our knowledge, that evaluates the moderating role of perceived stress change on the association between weight change and HbA1c change. Overall, our study showed that weight change was positively associated with HbA1c change in Puerto Rican women. Additionally, we found that change in perceived stress moderated the magnitude of the impact of weight

change on HbA1c change, where women with greater change in stress had greater HbA1c sensitivity to weight change. None of these associations were observed in Puerto Rican men.

Our results in women are in agreement with previous studies. For example, an analysis from The Nurses' Health Study showed that an increase in weight of 5 kg was associated with increased risk of diabetes in Latina women.[56] This is particularly important for Puerto Rican women residing in the mainland U.S. as they have the highest prevalence of obesity (50%) compared to other Latino groups.[10] In our sample of Puerto Rican men, weight change was not associated with change in HbA1c. This may be due, in part, because our sample size for men was smaller than that for women, limiting power to detect an association. In contrast, the DPP found results opposite to ours, as they documented greater reduction of glucose markers in men than in women.[63] They hypothesized that the difference in effect estimate between men and women was likely due to the greater load of diabetes risk factors observed in men (i.e., of older age, larger waist circumference and higher fasting glucose and blood pressure).[63] We observed the opposite; women in our study had a greater load of diabetes risk factors: they were less physically active than men, more likely to be on medications for depression or thyroid disease, and had higher BMI and perceived stress than men. Lastly, there was less variability in the distribution of HbA1c change in men than in women. Thus, all these factors may have contributed to the observed differences by sex and null results in men.

Our study provides evidence for our novel hypothesis of perceived stress as a moderator in the relationship between weight change and HbA1c change, where greater

change in perceived stress increases HbA1c sensitivity to weight change in women. The aforementioned longitudinal study that evaluated the association between weight gain and diabetes incidence in women did not evaluate the moderating role of stress in this association, but did document ethnic differences, which was higher for Latina women than for non-Latina white women.[56] Latinos, especially low-income Latinos, encounter numerous stressors throughout their life,[52,53] which may contribute to observed differences by race/ethnicity, but studies are needed to confirm this hypothesis. Data from the Whitehall Study II provides indirect support for our findings, as they found that work stress was positively associated with diabetes diagnosis in obese, but not in non-obese, women.[51]

Intervention studies also provide indirect support for our finding that stress is associated with greater HbA1c sensitivity to weight change.[58,60–62] For example, compared to the DPP,[58] intervention studies in low-SES communities show equivalent or greater improvement in HbA1c, with lower amounts of weight loss.[60–62] Because low-SES is a lifelong stressor associated with numerous other stressors (i.e., discrimination, poverty, violence),[107] these samples may have higher stress levels than those in the DPP, resulting in greater HbA1c sensitivity to weight loss. However, more longitudinal studies are needed to understand the moderating role of stress in the association between weight change (both weight loss and weight gain) and change in HbA1c. In our sample of Puerto Rican men, there was no significant interaction between weight change and change in perceived stress on HbA1c, although our sample size limited power in men. However, documented differences by sex in the perception of stressful live events,[108] where women

are more likely to report greater distress after a stressful event, coupled with the greater impact of stress on health observed for women compared to men,[26,27] may also explain our non-significant results in men.

The mechanism behind the interplay between stress and weight change on HbA1c change is complex, but the allostatic load (AL) framework may help understand it. AL explains how chronic stress leads to dysregulation of the body's regulatory systems.[15,28] In this process, chronic stress leads to dysregulation of the hypothalamic pituitary-adrenal axis (HPA), the sympathetic nervous system (SNS) and the immune system, as represented by dysregulation of primary AL markers (i.e., cortisol, epinephrine, norepinephrine, DHEAS and C-reactive protein). Chronic dysregulation of these primary markers leads to dysregulation of other secondary AL markers, such as hyperglycemia, which in turn lead to development of chronic diseases, like diabetes (tertiary AL marker). Thus, it is possible that individuals experiencing greater weight change concurrent with increase in perceived stress, may be more metabolically sensitive to changes in weight due to physiological changes occurring in response to chronic stress. However, more longitudinal studies are needed to confirm our findings. Our results also show that decreases in perceived stress < -5 units did not affect weight change sensitivity to HbA1c. Although stress reduction interventions have been shown to reduce some markers of AL (i.e.,cortisol,[109] HbA1c[110] and blood pressure[110]), it is unknown if it has the same impact on other parameters of the stress response and overall AL, and they have not been tested in Puerto Ricans. Thus, longitudinal studies that evaluate if reduction in perceived stress

leads to reduction in the entire physiological stress response and in improvement of glucose parameters are needed in this minority group.

Our findings that weight change and change in perceived stress have a compound effect on HbA1c suggest targets for diabetes prevention in Puerto Rican women. For example, physicians could monitor changes in weight and stress (with simple instruments like the Perceived Stress Scale), with intervention in high risk patients. However, more longitudinal studies are needed to confirm our findings and to elucidate novel strategies for diabetes prevention.

Our results need to be considered with several limitations. First, weight was measured at baseline and at 2 years after baseline. It is possible that participants had other weight change patterns that we were not able to account for during that period of time (for example, rapid weight loss and further weight regain), which may have implications on HbA1c.[111] Our measure of perceived stress only assessed stress levels in the prior month of the study visit, thus, participants may have experienced other trajectories in stress change that we were not able to account for. However, this is the first study, to our knowledge, that uses longitudinal data to evaluate how stress affects HbA1c sensitivity to weight change. In addition, our study was limited to Puerto Ricans residing in the Greater Boston area, thus it may not be generalizable to other Latinos. However, Puerto Ricans experience disproportionately high prevalence of obesity and diabetes[10] and high stress burden.

In conclusion, weight change was associated with change in HbA1c, and change in perceived stress increased HbA1c sensitivity to weight change in Puerto Rican women,

but not men. More longitudinal studies are needed to confirm our findings and to understand the role of sex in this association.

**Table 4.1.** Baseline characteristics of participants in the Boston Puerto Rican Health Study by sex.

| | **Men n=220 (28.5%)** | **Women n=552 (71.5%)** |
|---|---|---|
| ***Demographics*** | | |
| Age, mean (SD) | 56.2 (8.2) | 56.0 (7.4) |
| <High School, n (%) | 138 (62.7) | 344 (62.3) |
| ***Experiencing Menopause*** | - | 433 (78.4) |
| ***Behavioral factors*** | | |
| Smoker, n (%) | | |
| Never | 71 (32.3) | 282 (51.1) |
| Former | 70 (31.8) | 146 (26.5) |
| Current | 79 (35.9) | 124 (22.5) |
| Physical activity score, mean (SD) | 33.2 (6.1) | 31.5 (4.1) |
| Diet quality score, mean (SD) | 67.0 (10.2) | 71.9 (9.8) |
| ***Medication Use,*** **n (%)** | | |
| Medications for hypertension | 87 (39.6) | 234 (42.4) |
| Medications for hyperlipidemia | 62 (28.2) | 154 (27.9) |
| Medications for depression | 39 (17.7) | 208 (37.7) |
| Medications for thyroid | 6 (2.7) | 55 (10.0) |
| ***BMI, mean (SD)*** | 28.9 (4.8) | 32.2 (6.6) |
| ***Weight (in kg),*** mean (SD) | | |
| Baseline | 81.3 (14.9) | 77.8 (17.2) |
| Change | 0.33 (4.69) | 0.28 (6.13) |
| ***Perceived Stress,*** mean (SD) | | |
| Baseline | 21.6 (9.0) | 24.7 (9.1) |
| Change | -0.87 (8.88) | -0.72 (8.97) |
| ***HbA1c,*** mean (SD) | | |
| Baseline | 6.16 (1.11) | 6.26 (1.00) |
| Change | -0.07 (1.16) | 0.03 (0.87) |

**Table 4.2.** Association between weight change and HbA1c change among women in the Boston Puerto Rican Health Study.

| | Model 1 | | | Model 2 | | |
|---|---|---|---|---|---|---|
| | ***β*** | **95% CI** | **p value** | ***β*** | **95% CI** | **p value** |
| Weight change | 0.033 | 0.023-0.043 | <0.001 | 0.035 | 0.025-0.045 | <0.001 |

Model 1 is adjusted for baseline HbA1c. Model 2 is adjusted for baseline age, education, diet quality, smoking, physical activity, menopause status, BMI, HbA1c, and medications for hyperlipidemia, hypertension, depression and thyroid disease.

**Table 4.3.** Interaction between weight change and perceived stress change on change in HbA1c among women in the Boston Puerto Rican Health Study.

| Perceived stress change | $\beta$ | 95% CI | p value |
|---|---|---|---|
| -25 | -0.008 | -0.042, 0.025 | 0.619 |
| -15 | 0.009 | -0.013, 0.030 | 0.443 |
| -5 | 0.025 | 0.013, 0.038 | <0.001 |
| 5 | 0.042 | 0.031, 0.054 | <0.001 |
| 15 | 0.059 | 0.039, 0.080 | <0.001 |
| 25 | 0.076 | 0.044, 0.108 | <0.001 |
| 35 | 0.093 | 0.049, 0.137 | <0.001 |

Model is adjusted for baseline age, education, diet quality, smoking, physical activity, menopause status, BMI, HbA1c and medications for hyperlipidemia, hypertension, depression and thyroid disease. p value interaction term =0.006; p value LR test: 0.006.

# CHAPTER V:
# DISCUSSION AND CONCLUSIONS

## Summary of findings

The overall goal of this dissertation was to examine how stress is associated with metabolic dysregulation (in the context of diabetes) in Puerto Ricans, a population at high risk of diabetes and with great burden of physiological and psychological stressors. The conceptual model of this dissertation was informed by the AL framework, which conceptualizes that chronic physiological and psychological stress lead to disease development (i.e., diabetes) through a cascade of events (i.e., primary and secondary AL markers).[15,16] Thus, using the AL model as a conceptual framework, this dissertation evaluated physiological/psychological stress and the AL response in its stages, and the effect of psychological stress in associations between markers within the same AL stage. Additionally, given the documented sex differences in diabetes prevalence and in stress,[8,9,26,27] this dissertation explored the role of sex in the AL model. Using the Boston Puerto Rican Health Study data, the main objectives of this dissertation were to: (1) examine the relationship between GL and a composite score of primary markers of AL in men and women, (2) evaluate the interaction between perceived stress and GL on HbA1c in men and women and explore if a composite score of primary markers of AL mediates this interaction, and (3) confirm the association between weight change and change in HbA1c on men and women and evaluate if changes in perceived stress moderates this association.

Overall, the first aim of this dissertation showed that, in women, increase in dietary GL over a period of 2 years was associated with small increases in a composite score of primary AL markers. We did not observe this association in men. Due to the traditional Puerto Rican diet being high in GL, GL may be contributing to dysregulation of HPA axis, SNS and the immune system (as represented by primary AL markers) and thus influencing initiation of AL in Puerto Rican women.

The second aim of this dissertation found that concurrent psychological (perceived stress) and physiological (GL) stress may have a compound effect on HbA1c in women, but not in men. This interaction was not mediated by primary AL markers. These results partially confirm the AL model. Women with concurrent stressors had higher concentrations of a secondary AL marker (HbA1c), however findings did not confirm the AL chain of events, given that a composite score of primary AL markers did not mediate this association.

Lastly, the third aim of this dissertation found that increase in weight over 2 years was associated with increase in HbA1c in women but not in men. This aim also showed that women with increases in perceived stress over 2 years had higher HbA1c sensitivity to weight change. These results confirm the hypothesis that complex associations occur between AL markers within the same stage (secondary AL markers: weight change and HbA1c change) and the concept that stress influences disease risk through physiological changes.

In summary, using the AL model as framework, this dissertation showed that physiological and psychological stress were associated with physiological dysregulation

and glucose metabolism in Puerto Rican women. Thus, these finings suggest that both physiological and psychological stress may increase risk of diabetes in Puerto Rican women by rendering them to a greater metabolic disadvantage. This study also showed that these associations were not present in men. Thus, sex differences in the AL model may exist.

## Study Strengths and Limitations

Findings from this dissertation should be considered taking the following strengths and limitations in mind. GL, the exposure for Aims 1 and 2, was calculated from a FFQ and relied on self-reported dietary intake, thus responses may be biased due to social desirability and recall bias. Nonetheless, the FFQ used in the BPRHS was specifically modified and adapted for the Puerto Rican population and has been validated against 24hr-recalls.[73] Although a strength of this dissertation is the availability of longitudinal data to study changes in stress (i.e., psychological and GL) and physiological changes (i.e., primary AL markers, weight and HbA1c), one limitation specific to Aims 1 and 3 is that multiple trajectories (in GL, weight and perceived stress) may exist throughout the 2-year period but we were not able to evaluate them given that changes were calculated with two time points. Another limitation is that our sample was largely comprised of women (70% women), thus a limitation present throughout this dissertation work is the small sample size for men, which may have decreased statistical power to detect associations. However, our findings that physiological and psychological stressors were not associated with physiological dysregulation and glucose metabolism in men, are supported by studies showing that women are more vulnerable than men to the effects of

stress on health.[26,27,45,51] Lastly, another limitation of this dissertation is the limited generalizability of its findings. This study focused on Puerto Ricans residing in the Greater Boston area. Because this population faces unique socio-cultural stressors, have poorer intake of foods low in GL (i.e., vegetables and fiber) than other Latino groups,[112] and have amongst the highest prevalences of obesity and diabetes,[10] our findings may not be generalizable to other Latino groups. However, this limitation is also a strength given that Puerto Ricans experience considerable diabetes disparities.

**Discussion and Future Research Directions**

Diabetes continues to be a public health problem and its prevalence has been steadily increasing during the past 20 years.[1,2] This is even a greater burden in Puerto Rican men and women as this ethnic group experiences high prevalences of pre-diabetes and diabetes.[10,88,102] The AL model explains how chronic stress (unhealthy diet and psychological stress) influences disease through a cascade of events (represented by dysregulation of primary, secondary and tertiary AL markers).[15,28] With Puerto Ricans being characterized by having diets high in GL and being exposed to numerous psychosocial stressors,[52,53] it is imperative to understand how these stressors may affect disease risk at different AL stages.

Findings from this study underscore the potential role of GL as a physiological stressor in Puerto Rican women by contributing to dysregulation of the HPA axis, SNS and the immune system (primary AL stage). Emerging evidence has also linked carbohydrate consumption and dietary patterns high in GL with several individual primary AL markers (i.e., cortisol, norepinephrine and CRP).[39–43] Because chronic

dysregulation of the HPA axis, SNS and the immune system (represented by primary AL markers) is hypothesized to influence secondary markers of AL,[15,28] which include dysregulation of glucose metabolism, our results suggest a potential mechanism as to how GL may affect glucose metabolism and diabetes risk in women. Our null findings in men are consistent with studies reporting null associations between GL and diabetes in men.[38] Although more research is needed to understand how GL differentially affects physiological parameters in men and women, studies suggest that sex differences in carbohydrate and glucose metabolism may exist,[113,114] which may explain the observed sex differences in our study. Future studies evaluating the AL model should also examine the potential moderating role of sex. Overall, our findings are of great importance to Puerto Rican women given that the traditional Puerto Rican diet is characterized by foods high in GL. Future studies that confirm our findings and test interventions in women that aim to reduce GL in the context of the Puerto Rican diet are needed.

Our results also highlight the importance of studying the effect of concurrent stressors (high GL high perceived stress) on HbA1c in women. Previous studies had documented individual associations between GL/perceived stress and dysregulation of glucose metabolism.[35–37,47–50,97,98] Our study goes a step further and contributes to the current body of literature by evaluating the effect of experiencing two concurrent stressors, which is the living reality experienced by some women. Additionally, in an attempt to understand if concurrent stressors affect glucose metabolism through primary AL markers, we evaluated the mediating role of a composite score of primary AL markers (i.e., cortisol, epinephrine, norepinephrine, DHEAS and CRP) in this interaction.

Although our results did not confirm such hypothesis, it may be possible that it needs to be evaluated longitudinally, or that additional AL markers, like adiposity, are mediating this interaction. Studies of such nature are needed to truly understand how concurrent stressors are associated with dysregulation of glucose metabolism and diabetes risk in women. We did not observe any interactions between high GL and stress on HbA1c in men. This is consistent with studies of GL and diabetes,[38] as well as with hypothesis that women report greater perceived stress than men,[108] and are more metabolically sensitive to the effects of stress.[26,27,45,100] Future studies are needed to confirm our findings and to uncover specific stressors that are associated with physiological dysregulation and glucose metabolism in men.

Lastly, our findings indicate that stress may increase HbA1c sensitivity to weight changes in Puerto Rican women. We are not aware of any previous study that has evaluated the moderating role of stress in the association between weight change and HbA1c change, but longitudinal studies have reported varying estimates of this association (weight change and HbA1c change) between and within samples. More specifically, samples potentially experiencing more chronic stress (Latino ethnicity and low SES)[56,60–62] had higher effect estimates than samples with potentially less chronic stress (non-Latino Whites and higher SES),[56,58,59] which provide indirect support to our findings. Consistent with our results from Aim 1 and Aim 2, these associations were not observed in men, which may be due to the documented sex differences in the perception of stressful events[108] and to evidence suggesting greater impact of stress on health in women.[26,27] Our analysis did not test for potential mechanisms explaining how stress may

moderate the effect of weight change on HbA1c change, but the AL model may provide a plausible explanation. With perceived stress being associated with dysregulation of primary AL markers,[39,44–46] women experiencing increases in stress may be more metabolically sensitive to weigh changes due to the physiological dysregulation caused by chronic stress. These results may help identify women that are at high risk of disease and that may benefit the most from weight loss and stress management interventions.

Overall, the findings of this dissertation, guided by the AL model, show that stress (physiological and psychological) may increase risk of chronic diseases, like diabetes, in Puerto Rican women by rendering them to a greater metabolic disadvantage. Because AL is a dynamic network, our findings suggest that research that evaluates how chronic stressors are associated with physiological changes at each stage of the AL process (primary, secondary and tertiary AL markers) is needed to truly understand disease development and identify treatment targets and potential preventive interventions. In addition, our results suggest that women may be more vulnerable than men to the physiological effects of stress. Emerging research suggests that men and women may have different physiological responses to stress,[115,116] with women having a compromised cortisol feedback or delayed containment of the stress response.[115] Thus, studies that evaluate the role of sex in the AL process are also needed.

There are multiple future directions for the research presented in this dissertation. First, future studies should evaluate the role of sex in the AL model in order to improve our understanding of sex differences and to improve the AL model. Secondly, other dietary parameters (i.e., fats) can be evaluated as potential stressors affecting

physiological dysregulation and primary AL markers.[117,118] Thirdly, the models tested in this dissertation can be evaluated in other Latino groups at high risk of disease to examine if findings apply to other Latinos. In addition, these models could also be tested with other metabolic outcomes to evaluate if findings apply to other chronic diseases such as hyperlipidemia and hypertension. Lastly, other future next steps can include intervention studies that target GL and stress management to improve metabolic markers (i.e., primary AL markers and HbA1c) in women. In all, this dissertation provides insight into potential future studies that may help continue to understand how stress affects physiological dysregulation and uncover potential intervention targets to improve metabolic health in women.

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