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Cytokine profiling of young overweight and obese female African American adults with prediabetes

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ABSTRACT

Approximately 5–10% of subjects with prediabetes become diabetic every year. Inflammation is involved in the development of obesity-related type 2 diabetes (T2D). However, to date, the relationship between inflammation and prediabetes, defined by hemoglobin A1c (HbA1c) $\geqslant$5.7 and <6.5%, remains largely unexplored, especially in African Americans. Therefore, in this study we examined a comprehensive panel of 13 cytokines involved in the inflammatory response in overweight/obese subjects with prediabetes. A total of 21 otherwise healthy, overweight/obese, young adult African American females with prediabetes, together with 20 matched overweight/obese controls, were selected for this study. Plasma cytokines were assessed by multiplex cytokine profiling. Plasma concentrations of interleukin (IL)-5, IL-6, IL-7, tumor necrosis factor-α (TNF-α), and granulocyte–monocyte colony-stimulating factor (GM-CSF) were significantly higher in the prediabetic group, as compared to the control group (all $p < 0.05$). Plasma concentrations of all the other cytokines, interferon-γ (IFN-γ), IL-1β, IL-2, IL-4, IL-8, IL-10, IL-12p70 and IL-13, seemed to be elevated in the prediabetic group, but failed to reach statistical significances. Upon merging both groups, HbA1c was found to be positively correlated with IFN-γ, IL-1β, IL-2, IL-5, IL-7, IL-8, TNF-α and GM-CSF. This study demonstrates elevated levels of various pro-inflammatory cytokines in overweight/obese young subjects with prediabetes, which place them at higher risk of developing T2D and cardiovascular diseases. Our data also call for further investigations in animal models and population cohorts to establish the roles of a variety of pro-inflammatory cytokines in the early development of obesity-related T2D.

1. Introduction

Type 2 diabetes (T2D) associated with obesity is a rapidly growing public health problem and a major cause of morbidity and mortality. Prediabetes is characterized by an elevation of plasma glucose level above the normal range, but below that of diabetes, which can be identified as either impaired fasting glucose [IFG: 100 mg/dL (5.6 mmol/L) to 125 mg/dL (6.9 mmol/L)] or impaired glucose tolerance [IGT: 2-h values in the oral glucose tolerance test of 140 mg/dL (7.8 mmol/L) to 199 mg/dL (11.0 mmol/L)] [1]. The American Diabetes Association has recently defined prediabetes as hemoglobin A1c (HbA1c) between 5.7% and 6.4%, as HbA1c has higher repeatability versus fasting glucose, and does not require fasting [2].

Incidence of T2D rises steeply as HbA1c increases from the 5.0% to the 6.5% range [3]. Currently, approximately 79 million adults aged 20 years or older in the United States have prediabetes [4]. Epidemiological evidence clearly indicates the presence of diabetes-related complications in the prediabetic state [5–9]. The age-adjusted rates of diagnosed T2D and cardiovascular diseases (CVD) among adults 20 years or older are significantly higher in

Abbreviations: T2D, Type 2 diabetes; CVD, Cardiovascular diseases; IL, Interleukin; IFN, Interferon; TNF, Tumor necrosis factor; GM-CSF, Granulocyte-monocyte colony-stimulating factor.

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African Americans than in Caucasians, although the rates of prediabetes are similar (35%) in both ethnic groups [4]. These data indicate that a higher proportion of African Americans with prediabetes progresses to T2D than Caucasians. Moreover, incidence and mortality related to CVD is high among African–American females [10]. Although a recent study indicates significantly poorer preventive and riskier lifestyle behaviors in African Americans than in Caucasians [11], it is clear that, apart from altered glucose metabolism, other factors might also contribute to the observed differences in predisposition towards the development of T2D between these ethnic groups. As such, a better understanding of the pathogenic mechanisms underlying the progression from prediabetes to T2D, especially in African Americans, is of the utmost clinical importance.

Chronic inflammation plays an important role in the pathophysiology of T2D [1,12,13]. Bertoni et al. reported that higher baseline levels of interleukin-6 (IL-6), C-reactive protein (CRP), and fibrinogen were associated with increased incidence of T2D in a multiethnic American cohort [14]. To date, only few studies have examined pro-inflammatory parameters in prediabetic individuals. Gupta et al. observed increased levels of interferon (IFN)-γ, IL-6, tumor necrosis factor-α (TNF-α), and IL-1β in healthy Irish prediabetics compared to non-diabetics [15]. Increasing levels of CRP have been shown to be associated with progressively higher risk of IFG and IGT in Chinese subjects [16]. Plasma IL-6 was found to be elevated in Italian Caucasians with IGT and T2D [17]. Another study reported increased concentrations of TNF-α in Turkish females with IGT versus normal glucose tolerance (NGT) [18]. In contrast, Choi et al. [19] observed no significant differences in TNF-α and IL-6 concentrations in Korean women with IGT and NGT. Of interest, one study found markedly elevated plasma IL-8 concentration after glucose load in obese subjects with IGT as compared to NGT [20]. Moreover, hyperglycemia is known to increase the plasma concentrations of IL-6 and TNF-α within few hours and this effect is more distinct in individuals with IGT [21]. However, whether prediabetes, defined by HbA1c, is characterized by an increased presence of various pro-inflammatory cytokines has not been clearly demonstrated to date, particularly in African Americans, and therefore represented the main aim of this study. We profiled 13 cytokines, involved in the inflammatory response, in overweight/obese subjects, as compared with matched controls. In addition, we explored the relationships between HbA1c and pro-inflammatory cytokines in the entire cohort.

2. Materials and methods

2.1. Study population

Twenty-one apparently healthy young (18–45 years of age) adult African American females with prediabetes were recruited from the local communities of Augusta, Georgia and neighborhood areas. Overweight or obesity was defined by body mass index (BMI) $\geqslant$25.0 kg/m^2. Prediabetes was defined by HbA1c $\geqslant$5.7 and <6.5%. Twenty young adult African American females with HbA1c <5.7%, matched for BMI and age with these prediabetic cases, were selected as control subjects. Subjects were excluded if they were taking any prescription or over-the-counter medication including oral contraceptives, hormone replacement therapy, vitamins, herbals, or mineral supplements or had any acute or chronic medical illnesses. In addition, pregnant and breast-feeding females were also excluded. All subjects provided written informed consent and the study was approved by the Human Assurance Committee of the Georgia Regents University.

2.2. Anthropometrics, vitals, and blood collection

Height (cm) and weight (kg) measurements were obtained to calculate BMI. Waist circumference was measured by measuring tape at the level of umbilicus. Three blood pressure (BP) and heart rate (HR) readings, each one minute apart, were obtained by automated Dinamap monitor (Critikon, Tampa, FL) after 5–10 min of rest in the seating position with the use of an appropriate sized cuff wrapped on the non-dominant arm. The last two readings of BP and HR were averaged and recorded. Non-fasting blood samples were collected, frozen at −80 °C and were later used for the assessment of cytokine measurements.

2.3. HbA1c measurement

HbA1c was either measured from capillary blood by finger prick using Bayer's Professionals A1CNow + kit or from venous blood sample by Ion-Exchange Chromatography at the clinical laboratory of the Georgia Regents University. Both methods were certified by the National Glycohemoglobin Standardization Program (NGSP).

2.4. Plasma cytokine measurement

A panel of 13 pro-inflammatory cytokines [IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-13, TNF-α, and granulocyte–monocyte colony-stimulating factor (GM-CSF)] was assessed in triplicates in 50 μL plasma from the study subjects, using a highly sensitive cytokine bead assay (MILLIPLEX MAP High Sensitivity Human Cytokine Panel – Premixed 13 Plex, EMD Millipore) [22,23]. This assay has a high sensitivity, typically with a detection limit in the range from 0.01 to 0.48 ng/L. Data obtained using this cytokine bead assay technology have been shown to be highly reproducible and to be correlated well with values obtained using classical Enzyme-linked immunosorbent assay [24,25].

2.5. Statistical analyses

Descriptive statistics for continuous variables are presented as mean ± standard error of the mean. Continuous data were checked for normality using the Shapiro–Wilk test and were log transformed when needed. Group differences for age, anthropometrics, BP, and cytokines were determined by independent-t test if data were normally distributed or by Mann–Whitney *U* test otherwise. Associations of HbA1c with cytokines were assessed by Pearson's bivariate correlation coefficients in the total population. All analyses were performed using SPSS software (version 19.0; SPSS Inc., Chicago, IL) and statistical significance was set at $p < 0.05$.

3. Results

3.1. Clinical characteristics of study subjects in control and prediabetic groups

As demonstrated in Table 1, there were no statistical differences in age, weight, BMI, systolic BP, HR, or waist circumference between the two groups (all $p > 0.05$). However, the mean diastolic BP was found to be significantly higher in the prediabetic versus control group ($p = 0.05$).

3.2. Comparison of pro-inflammatory cytokines between both groups

We compared plasma levels of a panel of cytokines associated with inflammation, including IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-13, TNF-α and GM-CSF, between apparently healthy overweight/obese African American adult females