Sex-specific Relationships Between IL-3, IL-7 and Lipids in African Americans

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Abstract

Purpose: Obesity, a complex chronic disorder characterized by the enlargement of adipose tissue, has a multifactorial etiology. Adipose tissue is now recognized as an active tissue in the regulation of inflammation. Sex chromosome genes and hormones influences immune responses between males and females. Inflammation is rampant in obesity due to the expansion of visceral adipose tissue leading to insulin resistance resulting in type-2 diabetes (T2D). Differences in sex may lead to varied immune responses to T2D.

Methods: A total of 116 serum samples were collected from African Americans: 68 women and 48 men. All participants had a BMI > 30. This group consists of 49 normal HbA1c and 71 high HbA1c participants. This study was designed to determine the impact of current circulating glucose on current serum IL-3 and IL-7 levels.

Results: Serum cytokine levels are influenced by circulating high glucose and it varies based on sex. We found in women, IL-3 and IL-7 levels were upregulated 1.7-fold in the presence of high circulating glucose. In men, IL-3 levels were downregulated 1.5-fold and IL-7 levels downregulated 1.3-fold in the presence of high circulating glucose. IL-3 and IL-7 serum levels are also correlated with several lipid parameters.

Conclusion: IL-3 and IL-7 are members of a complex network of cytokines that play a role in chronic inflammation. Inflammatory signaling impact several diseases including obesity, T2D, atherosclerosis and dyslipidemia. A better understanding of the pathological signaling of cytokines will help facilitate our understanding of inflammation in these diseases.

Introduction

Obesity, a complex chronic disorder characterized by the enlargement of adipose tissue, has a multifactorial etiology, involving genetics, hormones, diet, and environment. Adipose tissue was considered as an inert tissue responsible for energy storage; however, it is now recognized as an active tissue in the regulation of inflammation [1]. Obesity is associated with alterations in immunity in which there are elevated levels of circulating pro-inflammatory cytokines. As visceral adipose tissue (VAT) expands, macrophages are recruited to the tissue, leading to the production and release of several adipokines and cytokines. This includes leptin, adiponectin, resistin, and visfatin, as well as interleukin (IL)-4, interferon (IFN)- γ , tumor necrosis factor (TNF)- α , IL-6, and several others [2, 3]. Pro-inflammatory molecules produced by VAT have been implicated as an active participant in the development of metabolic disease such as type 2 diabetes (T2D) and cardiovascular disease (CVD) [4].

Obesity increases cardiovascular risk by increased fasting serum triglycerides (TG), high LDL cholesterol, low HDL cholesterol, elevated blood glucose and insulin levels. These conditions are mainly developed due to excessive caloric intake and high fat diet (HFD). Serum lipid variances are common attributes of metabolic syndrome and may be correlated to a pro-inflammatory gradient. An important connection between obesity, metabolic syndrome and dyslipidemia, may be the development of insulin resistance

(IR) in tissues such as the liver. This leads to an influx of circulating fatty acids due to dietary sources, the breakdown of fat in the blood vessel and resistance to the anti-lipolytic effects of insulin [5]. All of these conditions result in increased circulating lipids and cholesterol particles in the blood. Entry and retention of LDL particles in the arterial wall trigger inflammatory signals, resulting in the expression of adhesion molecules by the endothelium and the local secretion of cytokines and chemokines. Ultimately, an increase in circulating lipids contributes to the accumulation of macrophages and other inflammatory cells in the subendothelial space [6, 7].

Interleukin-3 (IL-3) belongs to the β common cytokine family, in addition granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF) and IL-5. IL-3 plays a role in leukocyte development. This cytokine is largely used for steady-state hematopoiesis but is also produced at sites of inflammation and therein exert pathophysiologic effects. The β common chain family of cytokines regulate various inflammatory responses that promote the rapid clearance of pathogens but also contribute to pathology in chronic inflammation. A previous study indicated that an HFD induced central and peripheral high leukocyte count and circulating neutrophils levels in rats [8]. The HFD animals had a higher number of bone marrow cells, which had a greater capacity to produce IL-3 and G-CSF [8]. It appears that HFD has an indirect effect on the levels of IL-3. The increased circulation of leukocytes has been reported in obese individuals as well as individuals with features of metabolic syndrome, coronary artery disease and complications from T2D [9–12].

Interleukin-7 (IL-7) is a cytokine essential for cell survival and proliferation of both naïve and memory T cells [13]. IL-7 binds to interleukin-7 receptor alpha (IL-7R α) and γ_c , activating JAK/STAT and AKT signaling pathways. Non-hematopoietic and dendritic cells produce IL-7; moreover, normal adult human hepatic tissues produce IL-7 transcripts and proteins. A lack of IL-7R α in humans has, been known to exhibit severe combined immunodeficiency [14]. Polymorphisms in IL-7R are risk factors for several involved in excess immune and inflammatory responses. IL-7 is markedly increased in the serum of obese individuals. IL-7R is overexpressed in white adipose tissue (WAT) in obesity; a previous study measured the metabolic outcomes of IL-7R knockout (KO) mice on chow and HFD [15]. The expression of genes and proteins related to IR and inflammation were evaluated in WAT. They found that IL-7R KO mice exhibited significantly less weight gain and visceral fat compared to wildtype controls on both chow and HFD [15]. Overall, the IL-7R KO mice has less adipogenesis signaling, pro-inflammatory cytokine production and macrophage infiltration in the WAT. These results imply that HFD may activate pro-inflammatory production and release, including IL-7. IL-7 has also been implicated in the progression of atherogenesis by recruiting monocytes/macrophages to the endothelium [16]. Both innate and adaptive immunity have been suggested to modulate the rate of lesion progression. Atherosclerosis is characterized by the infiltration of immune cells and the presence of lipid-enriched macrophages in the arterial wall and is a major cause of CVD.

The immune system plays a major role in the development and progression of CVD and T2D. Among mammals, males and females differ in their innate immune responses, which suggests that some sex differences may be germline encoded. The number and activity of cells associated with innate immunity

differ between the sexes; males have high natural killer cells frequencies than females [17]. Neutrophils and macrophages have higher rates of phagocytosis in female than males [18]. Sex influences multiple aspects of adaptive immunity as well. The thymus plays an essential role in the development of the adaptive immune system by generating the peripheral T cell pool. Adolescent male rats have larger thymuses, greater thymocyte levels and a difference in distribution of thymocyte subsets in comparison to female rats [19, 20]. In addition, adult humans have sex differences in lymphocyte subsets: B cells, CD4 + T cells and CD8 + T cells. These differences are observed across multiple ethnic groups as well, including Europeans, Asians, and Africans [19–21].

Sex chromosome genes and hormones which includes estrogens, progesterone and androgens, influence differential regulation of immune responses between males and females. In addition, women have different sex steroid concentrations and X chromosome diploidy which plays a significant role [22]. Although infant and childhood males produce higher inflammatory responses than females, after puberty inflammatory responses are consistently higher in females than in males [23]. Sex differences in immune responses are altered throughout life and are influenced by both the age and reproductive status. As humans age, concentrations of sex steroids decline rapidly for females and more gradually in males. Thus, males in reproductive senescence tend to have an increased inflammatory response than females [24]. Immunological differences between sexes reflect hormonal, genetic and environmental effects on the immune system that can change throughout life in humans. Despite a growing body of evidence demonstrating sex-based differences in immune responses, fewer than 10% of immunology articles analyze data by sex in previously published papers [25].

Inflammation is rampant in obesity due to the expansion of VAT leading to IR in metabolic and peripheral tissue resulting in T2D. Inflammatory cytokines also play a major role in the progression of T2D and diabetic complications. This study was designed to investigate the influence of high HbA1c on IL-3 and IL-7 serum levels of obese, African American women and obese, African American men. African Americans are the most at risk population for obesity and T2D and would benefit greatly from this study. To eliminate obesity as a confounding factor of cytokine levels, both the control and experimental groups were obese for males and females. We also investigated the relationship between IL-3 and IL-7 levels and clinical metabolic parameters. Herein, we present data showing serum IL-3 and IL-7 levels increase in obese African American women with high HbA1c in comparison to control group. We also observe a decrease in serum IL-3 and IL-7 levels in obese, African American males with high HbA1c in comparison to control group. In addition, several clinical metabolic parameters are correlated with these key inflammatory cytokines.

Methods

Study Population

A total of 116 serum samples were collected from African Americans: 68 women and 48 men. All participants had a BMI > 30. This group consists of 49 normal HbA1c and 71 high HbA1c participants. We

define normal HbA1c as ≤ 6.5 and high HbA1c as > 7.0 regardless of diabetic status. This study was designed to determine the impact of current circulating glucose on current serum IL-3 and IL-7 levels. All participants were over the age of 40 and located in a rural northeastern county in North Carolina. Participants in both groups were on medications to regulate hypertension, cholesterol and blood glucose. Participants were fasting during blood collection. Blood samples were processed, and serum was collected and immediately frozen at -80°C until use. We cased matched the participants in this study based on age and BMI. We also made sure that every pair has at least a 2% difference in HbA1c. Informed written consent was obtained for all participants and the right to privacy was observed according to the North Carolina Central University Institutional Review Board approved protocol.

Cytokine/chemokine Quantification

Interleukin-3 and 7 were quantified using panel MILLIPLEX MAP Human Cytokine/Chemokine Multiplex Assay (Milliplex map kit, HCYTMAG-60 K; Millipore, USA) following the manufacturer's instructions. Analyte quantification from the reactions were obtained using Luminex Technologies and Luminex Xponent managed the data output. Milliplex analyst software (Version 5.1 Flex; Darmstadt, Germany) was used to determine the analyte concentration (pg/mL) using mean fluorescence intensity (MFI).

Patient Metabolic Parameter Quantification

Clinical metabolic parameters were determined using standard commercial kits administered by Laboratory Corporation of America.

Lipoprotein (a) Quantification

Lipoprotein (a) was quantified using LP(A) reagent (Kamiya Biomedical; KAI017; Seattle, Washington, USA) following the manufacturer's instructions. This test was used for the quantitative determination of human Lp(a) by immunoturbidimetric assay. Serum is mixed with anti-human Lp(a) antiserum, agglutination is caused by the antigen-antibody reaction. The turbidity is measure at 340 and 700 nm and the Lp(a) in the sample is quantitatively determined.

Statistical analysis

The data from the table were expressed as mean $\pm$ SEM values. The experimental and control groups were compared using unpaired t-test. The relationship between cytokines and clinical metabolic parameters was assessed by Pearson's correlation coefficient (r). Graph- Pad Prism software (Version 6.05; San Diego, CA, USA) was used for statistical analysis and graphical representation of the data. P-values for each assessment that are ≤ 0.05 , were noted as statistically significant.

Results

Cytokine expression plays a role in cholesterol and lipid metabolism. We quantified IL-3 and IL-7 serum levels in 45 obese, normal HbA1c participants and 71 obese, high HbA1c participants. The basic demographic and clinical metabolic parameters of all participants are shown in Table I. For women, there is no significant difference in age or BMI between the two groups. There is a significant difference in HbA1c ($p < 0.0001$) and fasting glucose levels in the serum ($P < 0.0001$). We also observed a significant difference in circulating VLDL cholesterol ($P = 0.005$), TG ($P = 0.019$) and sodium ($P = 0.008$) between the two groups of women. There was no significant difference between the other parameters measured in women. In men, there is no significant difference in age or BMI between the two groups. In men, there is no significant difference in age or BMI between the two groups. There is only a significant difference in HbA1c ($p < 0.0001$) and fasting glucose levels in the serum ($P = 0.01$).

Table I

Patient Characteristics	Women				Men			
	All Women	Normal HbA1c	High HbA1c	P-value Normal HbA1c vs. High HbA1c	All Men	Normal HbA1c	High HbA1c	P-value Normal HbA1c vs. High HbA1c
	(mean± SEM)	Women (mean± SEM)	Women (mean± SEM)		(mean± SEM)	Men (mean± SEM)	Men (mean± SEM)	
		N=34	N=34			N=11	N=37	
Age (years)	60.54 ±1.06	60.88 ±1.51	60.21 ±1.52	0.753	68.95 ±0.47	68.21 ±0.77	69.19 ±0.57	0.37
BMI (kg/m ²)	35.73 ±0.96	34.72 ±1.56	36.74 ±1.13	0.299	35.49 ±1.05	38.74 ±2.65	34.41 ±1.06	0.075
HbA1c (%)	7.76 ±0.26	5.94 ±0.05	9.58 ±0.28	<0.0001	8.03 ±0.29	5.94 ±0.13	8.73 ±0.31	<0.0001
Glucose Serum (mg/dL)	142.4 ±9.66	101.4 ±3.30	183.4 ±16.31	<0.0001	143.1 ±8.65	106.8 ±7.99	155.2 ±10.52	0.01
Total Cholesterol (mg/dL)	179.20 ±5.01	178.6 ±6.58	179.9 ±7.66	0.896	161.9 ±5.36	158.8 ±8.72	162.9 ±6.59	0.744
HDL Cholesterol (mg/dL)	55.13 ±2.52	59.85 ±4.41	50.41 ±2.24	0.062	45.75 ±2.21	40.58 ±2.77	47.47 ±2.76	0.18
LDL Cholesterol (mg/dL)	99.24 ±3.92	100.9 ±5.61	97.58 ±5.54	0.689	93.72 ±3.83	94.58 ±6.68	93.43 ±4.65	0.9
VLDL Cholesterol (mg/dL)	22.30 ±1.64	17.85 ±1.71	26.88 ±2.61	0.005	23.98 ±1.62	23.50 ±2.29	24.14 ±2.05	0.865
Triglycerides (mmol/L)	123.60 ±14.54	89.24 ±8.49	157.9 ±26.73	0.019	137.8±19.60	118.3±11.59	144.3 ±25.87	0.571
Creatinine (mg/dL)	0.89 ±0.04	0.81 ±0.02	0.96 ±0.07	0.076	1.08 ±0.03	1.06 ±0.06	1.08 ±0.04	0.728
BUN/Creatinine (Ratio)	17.38 ±0.69	17.44 ±1.09	17.32 ±0.86	0.933	14.50 ±0.51	14.08 ±0.83	14.64 ±0.63	0.647
Sodium (mmol/L)	140.20 ±0.34	141.1 ±0.38	139.4 ±0.51	0.008	140.1 ±0.37	140.8 ±0.72	139.9 ±0.44	0.283
Calcium (mg/dL)	9.48± 0.05	4.09 ±0.06	4.27 ±0.09	0.849	9.29± 0.05	9.32 ±0.11	9.28 ±0.06	0.73
Potassium (mmol/L)	4.18±0.05	9.49 ±0.08	9.47 ±0.06	0.092	4.33±0.05	4.25±0.08	4.36 ±0.06	0.375
C-Reactive protein*	6.38±1.08	4.94 ±1.05	8.45 ±2.101	0.149	11.62±1.08	4.21 ±1.05	15.07±11.24	0.522

* N=23 for normal HbA1c and N=16 for high HbA1c women

*N=7 for normal HbA1c and N=15 for high HbA1c

Serum cytokine levels are influenced by circulating high glucose and it varies based on sex. We found a difference in serum IL-3 levels in males ($P = 0.03$) and females ($P = 0.0007$) with high HbA1c (Fig. 1a and b). In women, IL-3 levels were upregulated 1.7-fold in the presence of high circulating glucose. In men, IL-3 levels were downregulated 1.5-fold. We found a difference in serum IL-7 levels in males ($P = 0.04$) and females ($P = 0.0007$) with high HbA1c (Fig. 1d and e). In women, IL-7 levels were upregulated 1.7-fold in the presence of high circulating glucose. In men, IL-7 levels were downregulated 1.3-fold. When sexes are combined, there is no difference in serum IL-3 and IL-7 levels in the presence of high glucose (Fig. 1c and f). Serum IL-7 levels are correlated with body mass index (BMI). In women with normal HbA1c, we observed a positive correlation between serum IL-7 and BMI ($R = 0.51$, $P = 0.05$) (Fig. 2a). We observed a negative trend in normal HbA1c men, between serum IL-7 levels and BMI ($R = 0.52$, $P = 0.08$) (Fig. 2b). IL-3

is negatively correlated with BMI in both women ($R = -0.37$, $P = 0.04$) and men ($R = -0.52$, $P = 0.02$) with high HbA1c (Fig. 3a and b).

The liver macro-environment influences T2D through glucose, lipid and cholesterol metabolism. C-reactive protein (CRP) is expressed in the liver in response to inflammation. We found that all women participants have a positive correlation with CRP and IL-3 ($R = 0.37$, $P = 0.05$) (Fig. 4a). In addition, we show a positive correlation with CRP and IL-7 ($R = 0.69$, $P = 0.0002$) in all women participants (Fig. 4b). In all men participants, CRP was not correlated with IL-3 or IL-7 (Fig. 4c and d). IL-3 has no correlation with VLDL or TG in women with normal HbA1c (Fig. 5a and b). However, in men with normal HbA1c, IL-3 is positively correlated with VLDL ($R = 0.56$, $P = 0.05$) and TG ($R = 0.56$, $P = 0.05$) (Fig. 5c and d). IL-7 has a positive correlation with VLDL ($R = 0.53$, $P = 0.03$) and TG ($R = 0.52$, and $P = 0.04$) in women with normal HbA1c (Fig. 6a and b). IL-7 has no correlation with VLDL and TG in men with normal HbA1c Fig. 6c and d). Lp(a) is positively correlated with IL-3 in women ($R = 0.45$ and $P = 0.03$) and men ($R = 0.51$, $P = 0.01$) (Fig. 7a and b).

Discussion

IL-3 and IL-7 are members of a complex network of cytokines that play a role in chronic inflammation. Inflammatory signaling impact several diseases including obesity, T2D, atherosclerosis and dyslipidemia. A better understanding of the pathological signaling of cytokines will help facilitate our understanding of inflammation in these diseases. When the quantity or interval of inflammation is dysregulated due to obesity, patients are at risk for CVD. Diabetic and cardiovascular complications are prominent in obese populations due to the presence of chronic, low-grade inflammation. African Americans have the highest prevalence of obesity and T2D. This population is the least studied and could greatly benefit from these findings. We designed a study to investigate the influence of high HbA1c on serum IL-3 and IL-7 levels of obese, African American women and obese, African American men. We eliminated obesity as a confounding influence on cytokine levels by selecting obese participants in the control and experimental groups, hence answering the question of HbA1c influence alone. We also wanted to determine the correlation between IL-3 and IL-7 with clinical metabolic parameters as they relate to high blood glucose. Despite a growing body of evidence demonstrating sex-based differences in immune responses, they are least studied.

Obese, African American women have an increase in serum IL-3 and IL-7 resulting from high HbA1c (Fig. 1a and d). Obese African American women have a different inflammatory response to circulating high glucose than men. Interestingly, obese, African American men have a decreased inflammatory response to circulatory high glucose. We observed a decrease in serum IL-3 and IL-7 (Fig. 1b and e) in African American men. Although men have a decrease immune response to high circulating glucose, the quantity of serum IL-3 and IL-7 is approximately 2-fold higher than women. In previous research, women have been reported to have a decrease inflammatory response in comparison to men due to a rapid decline in sex-steroid during reproductive senescence [24]. Previously, researchers often combine men and women into control and experimental groups [26–28]. When we combine men and women in the

control and experimental, there is no difference between participants with normal and high HbA1c (Fig. 1c and f). We show that sex is a biological variable that affect immune response of IL-3 and IL-7. Sex is also a contributing factor to physiological response to circulating high glucose.

In addition to sex differences in immunological response of IL-3 and IL-7, obese African Americans show a difference in correlation with several clinical metabolic parameters. Obese women and men with normal HbA1c show inverse correlations with BMI and IL-7. Women with normal HbA1c have a positive correlation between BMI and IL-7 and normal HbA1c men have a negative correlation between BMI and IL-7 (Fig. 2a and b). BMI trends suggest that majority of the increase in the prevalence of T2D is resulting from the increased prevalence of obesity. In fact, 85.2% of people with T2D are overweight or obese [29]. Given that obesity is highest in African American women, one would expect this group to be at the greatest risk for developing T2D. Based on our data, it appears that an increase in BMI and HbA1c increases the serum levels of IL-7 in African American women only; men show the inverse effect. Our data suggests that African American women follow the proposed mechanism that obesity and expansion of VAT causes low-grade inflammation leading to IR and T2D. As BMI and HbA1c increase, African American men have lower levels of serum IL-7. Women and men with high HbA1c exhibited similar negative correlations with BMI (Fig. 3a and b). As BMI and HbA1c increase, African American men have lower levels of serum IL-3. Serum IL-3 levels show opposite correlation with BMI in comparison to IL-7 in women. IL-3 may not be as prominent in the expansion of VAT as IL-7 in women. BMI and HbA1c are prominent risk indicators for metabolic disease; however separating groups by sex shows that the impact on inflammation could be interpreted differently.

C-reactive protein, an acute-phase protein originating from the liver, increases in the serum in response to inflammation. We found that all women participants had a positive correlation with CRP and IL-3 as well as IL-7 (Fig. 4a and b). There was no correlation with CRP and IL-3 or IL-7 in men (Fig. 4c and d). Previous research has reported that women generally have higher CRP levels than men, but the mechanisms for this observation are unknown [30–32]. CRP levels may correlate with adiposity in women greater than men [31]. Previous reports observed a stronger correlation between total fat mass and CRP levels in women compared with men [33]. Researchers have also previously reported sex differences in the relationship between BMI and CRP [31, 34–36]. However, BMI may not be the best measure of adiposity because it can inaccurately quantify body fat. VAT has been shown to be more metabolically active in individuals with abdominal obesity and have an increased risk of developing IR or metabolic syndrome. Women generally have more body fat than men for the same BMI value [37]. This can account for the correlation between CRP levels and IL-3 and IL-7 seen in women. Sex differences in CRP correlations may be based on the quantity and regional distribution of adipose tissue in various compartments, including abdominal depots which are more prominent in women.

Dietary fatty acids increase inflammatory markers such as CRP, soluble adhesion molecules, IL-6, TNF- α [38–39]. High serum lipid levels are a risk factor for CVD. Large lipoproteins in addition to TG-rich lipoproteins can infiltrate the vascular wall. Mounting evidence suggests that all TG-rich lipoproteins stimulate cytokine expression in circulating monocytes [40]. VLDL stimulates monocyte adhesion to

endothelial cells and expression of inflammatory genes in macrophages. We found several correlations with IL-3 and IL-7 with VLDL as well as TG that differed by sex. In obese women with normal HbA1c, IL-7 is positively correlated with VLDL and TG (Fig. 6a and b). There is no correlation with IL-7 and VLDL or TG in men with normal HbA1c (Fig. 6c and d). In a previous study, female mice that constitutively over-express IL-7 displayed glucose intolerance and insulin resistance, traits that were accompanied by abnormal accumulation of adipose tissue in both humans and animals [41–42]. Previous reports describe a sexual dimorphism in mice that affect adipose tissue accumulation, gene expression, and insulin sensitivity with the overexpression of IL-7 [43–45]. Sex hormones have been compellingly described to alter adipocyte biology and to influence obesity-related co-morbidities, such as IR [46–48].

Obesity can be classified as an inflammatory disease; previous studies show that male and female mice differ in the inflammatory response resulting from diet-induced obesity [49]. Sex differences in HFD-induced inflammatory response increases adipose tissue infiltration with M1 macrophages in males, but M2 macrophages in females. Males typically have increased levels of inflammatory cytokines compared to females [50]. Both male and female participants had a positive correlation between Lp(a) and IL-3. Fatty acids released from large lipoproteins can stimulate both vascular cells and circulating monocytes. It is likely that fatty acids released from TG-rich lipoproteins contribute to atherogenesis. Fatty acids influence serum lipoprotein levels and may stimulate numerous atherogenic cellular functions. In normal HbA1c men, IL-3 is positively correlated with VLDL and TG (Fig. 5c and d). There is no correlation with IL-3 and VLDL or TG in women with normal HbA1c (Fig. 5a and b). Mounting evidence demonstrates that genetic variations in the Y chromosome are associated with lipoprotein profiles in men and mice [51–53]. It is well established that the excessive consumption of an HFD results in obesity and an increase in leptin concentrations, which triggers a chronic inflammatory condition that is associated with a high white blood cell count. Male Wistar rats fed an HFD for 12 weeks. exhibited leukocytosis and neutrophilia with increased CRP, leptin, cholesterol and TG concentrations [8]. In addition, the HFD bone marrow cells had a higher capacity to proliferate and differentiate into granulocytic cells which produce IL-3. This data suggests that males with HFD can induce leukocytosis and neutrophilia leading to alterations in serum IL-3 levels.

Conclusion

In this study, we examined the effect of circulating high glucose on serum IL-3 and IL-7 levels in obese, African American men and women. In addition, we investigated the correlation between pro-inflammatory cytokines IL-3 and IL-7 with markers of lipid metabolism. In previous research, elevated levels of pro-inflammatory cytokines have been reported in obese, African American women [54–55]. Our results show that serum IL-3 and IL-7 increase as HbA1c increases in obese, African American women. However, in obese African men, serum IL-3 and IL-7 decreases as HbA1c increases. In addition, African American men had approximately 2-fold higher IL-3 and IL-7 levels than women. These results highlight potential sex differences in pro-inflammatory cytokines levels in the progression of T2D. Our correlative data also suggests sex differences in serum IL-3 and IL-7 levels as possible new regulators of lipid metabolism humans.

Sex-based differences in the activity of the innate and adaptive immune responses likely have evolved through a process of convergent evolution which have sex-specific effects on immune function. Previous reports [56] highlight the significance of personalized medicine for different racial subgroups. This study shows that sex should also be taken into consideration when treating T2D and dyslipidemia. This study also highlights two novel inflammatory biomarkers for metabolic diseases in African Americans. Future studies must continue to identify the specific factors mediating sex differences in the immune responses in T2D. Our long-term goal is to define immune response mechanisms that will contribute to precision treatments for immune mediated diseases such as T2D, atherogenesis and dyslipidemia.

Declarations

Availability of data and material

Please contact author for data requests.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Author's Contribution

AW was responsible for multiplexing, sample management, data management, data interpretation, and drafting the manuscript. NG was responsible for experimental design and sample acquisition. CWD was responsible for experimental design. KSK was responsible for experimental design, data interpretation, manuscript editing. KSK is the Guarantor.

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Figures

Figure 1

Serum cytokine levels in normal and high HbA1c individuals. The number of individuals fluctuate due to some values registering below threshold. Cytokine levels were measured using magnetic beads from 30-plex immune assay from Millipore Sigma. *significant at $p<0.05$; ** significant at $p<0.005$; *** significant at $p<0.001$; **** significant at $p<0.0001$

Figure 2

The correlation between BMI and IL-7 in women and men with normal HbA1c. Cytokine levels were measure using magnetic beads from 30-plex immune assay from Millipore Sigma.

Figure 3

The correlation between BMI and IL-3 in women and men with high HbA1c. Cytokine levels were measure using magnetic beads from 30-plex immune assay from Millipore Sigma.

Figure 4

The relationship of CRP with inflammatory cytokines in all participants. Cytokine levels were measure using magnetic beads from 30-plex immune assay from Millipore Sigma.

Figure 5

The relationship of lipids with IL-3 in women and men with normal HbA1c. Cytokine levels were measure using magnetic beads from 30-plex immune assay from Millipore Sigma.

Figure 6

The relationship of lipids with IL-7 in women and men with normal HbA1c. Cytokine levels were measured using magnetic beads from 30-plex immune assay from Millipore Sigma.

Figure 7

The relationship of Lp(a) with IL-3 in all participants. Cytokine levels were measure using magnetic beads from 30-plex immune assay from Millipore Sigma.