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Original article

Chemokines and their association with body mass index among healthy Saudis

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ABSTRACT

Obesity is a chronic disorder that is associated with body mass index (BMI) of greater or equal to 30 kg/m². The prevalence of obesity in the Kingdom of Saudi Arabia (KSA) is increasing at an alarming rate and is expected to reach 41% in men and up to 78% in women by 2022. Since chemokines are associated with involuntary weight loss, the objective of this study was to elucidate their association with BMI among Saudis. A questionnaire was used to collect information about diet, health conditions, and demographics from 15 men and 16 women who participated in the study. BMI was calculated based on clinical measurements and participants were classified according to their BMI category as: normal, underweight, overweight, or obese. Serum samples were collected for a multiplex assay using the Human Chemokine Magnetic 30-plex panel. The serum concentration of either the monokine induced by gamma interferon (MIG) or the CXC-motif chemokine ligand 9 (CXCL-9) was significantly increased in obese men ($P = 0.0194$) and women ($P = 0.043$) as compared to underweight men and women, respectively. However, the serum levels of other chemokines were not significantly different among the groups. We found that MIG levels are differentially regulated in serum, based on individuals' BMI.

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1. Introduction

Obesity is a chronic disorder associated with body mass index (BMI) of 30 kg/m² or more. It is associated with an accumulation of body fat, which is directly or indirectly associated with adverse

health effects (National Heart Lung and Blood Institute NIOHN, 1998). In addition to its impact on individuals' quality of life, obesity is a major public health concern with social and economic implications in many countries. In the United Kingdom, it is estimated that approximately 60% of males and 50% of females will be obese by 2050 (Lobstein, 2007). Alarmingly, child obesity in the United Kingdom is similarly increasing and by 2050 nearly 60% of boys aged between 6 and 10 years will be obese compared to the girls (The NHS Information Center LS, statistics-on-obesity-physical-activity-and-diet.pdf,2010). In the United States, obesity has been estimated to cost about \$100 billion annually due to both management of co-morbid diseases and significant challenges faced by the obese, especially the social stigma leading to poor outcome in education and hence increase in unemployment (Northstone et al., 2012; Panuganti and Lenehan, 2018). The prevalence of obesity in the Kingdom of Saudi Arabia is projected to increase from 12% in men and 21% women in 1992 to 41% in men and 78% in women by 2022 (Al-Quwaidhi et al., 2014).

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Common health disabilities and disorders associated with obesity frequently include learning disabilities, depression, asthma, sleep disorders in the young and ischemia, infarction, hypertension, arthritis, stroke, diabetes, and several types of cancers in adults (Northstone et al., 2012; Sepulveda et al., 2018; Villareal et al., 2005). Insights into the underlying factors and mechanisms involved in obesity will help in developing the means to limit the associated morbidity and mortality.

Certain chronic diseases are associated with underlying inflammation, which is in turn intrinsically related to the progression to obesity (Gregor and Hotamisligil, 2011). Pro-inflammatory mediators like tumor necrosis factor alpha (TNF- α), several interleukins (IL) such as IL-1b, IL-6, IL-8 and the nuclear protein high mobility group box-1 (HMGB1), are implicated in several processes related to Obesity. HMGB1 is one of the important mediators known to be involved in signaling by the Toll-like receptors as well as through receptors for advanced glycation end products (RAGE) (Mraz and Haluzik, 2014). Other inflammatory biomarkers can be modulated by obesity including C-reactive protein (CRP), TNF- α , IL-1 α , IL-4, IL-10, and transforming growth factor-beta-1 (TGF- β 1), which shifts the balance in the cytokine to an “anti-inflammatory” status (Billon and Dani, 2012; Wajchenberg, 2000). Thus, it can be concluded that inflammation is an important milieu for weight gain.

Cytokines and chemokines are secreted in response to unfavorable stimuli including excess nutrients and causing the recruitment of antigen-presenting cells and T and B lymphocytes, thus creating an inflammatory response with predisposition to obesity (Gangemi et al., 2012; Lassenius et al., 2011). There are two types of adipose tissues: the white (WAT) and the brown (BAT) adipose tissues. WAT stores energy as triglycerides, while BAT utilizes lipids through adaptive thermogenesis thus maintaining the energy balance (Billon and Dani, 2012). Interestingly, the subcutaneous WAT is understood to be involved thermoregulation and offer protection while visceral WAT is linked to metabolic diseases and obesity (Wajchenberg, 2000). Pro-inflammatory cytokines, particularly interleukins (IL-6, IL-1 β), CRP, and TNF- α , are expressed in WAT (Bastard et al., 2006). In addition, the monocyte chemo-attractive protein-1, M1 macrophages and T- and B-cells have been reported to be increased in obesity (Odegaard and Chawla, 2013).

Chemokines are small proteins (8–10 kDa) that help recruit immune cells to sites of inflammation. To date, the chemokine system includes 50 chemokines with 20% to 50% sequence homology with each other, and 20 chemokine receptors belonging to the seven-transmembrane G-protein-coupled receptor family (Ota, 2013; Xu et al., 2015; Yao L and Heuser-Baker J, 2014). Notably, the chemokine receptors are promiscuous and thus bind more than one chemokine, demonstrating a high level of functional redundancy (Xu et al., 2015). Interestingly, low-grade chronic inflammation of the mediobasal hypothalamus is associated with obesity and is mediated by production of pro-inflammatory cytokines IL-6, IL-1 β , and TNF- α (De Souza et al., 2005). Given the complex relationships of chemokines/cytokines in the context of obesity, we sought to evaluate the role of chemokines in relation to BMI among healthy Saudi men and women in a study conducted at King Abdulaziz University in Jeddah, Saudi Arabia.

2. Materials and methods

2.1. Study population

The study was approved by the ethical Committee at King Abdulaziz University (Reference number 361-14). One hundred ninety adults were randomly recruited to participate in the study. Of these, only 117 satisfied both the inclusion and exclusion criteria

and were enrolled (56 women and 49 men). Out of those, a total of thirty one healthy adults 16 women and 15 men were selected as representative to be included in this study.

2.2. Study design

A cross sectional study design was employed to conduct the survey. Individuals were recruited from healthy adults accompanying relatives in primary health centers and hospitals in Jeddah and Makkah. All participants completed a pre-designed questionnaire covering their socio-demographic information, medical history, lifestyle and dietary practices. People were included if they were ≥ 18 years of age, apparently healthy, and not on any medications that might affect weight or sleeping habits. Recruits were interviewed by trained medical students using a designed structured questionnaire that included demographic information, as well as specific questions on dietary habits, patterns and intake, in addition to specific questions on sleeping patterns. Anthropometric measurements and blood pressure were taken using calibrated instruments.

2.3. General health indicators and anthropometric measurements

The height of each participant was measured to the closest 0.5 cm, using a stadiometer (Detecto, Webb City, MO, USA). The weight of lightly clothed participants was measured to the nearest 0.5 kg, using a portable calibrated scale (Omron BF511, Beringe, Netherlands). Blood pressure was measured using a mercurial blood pressure monitor (Kawamoto Corporation, Osaka, Japan), and heart rate was also recorded. Anthropometric measurements including waist circumference, hip circumference, and neck circumference were measured, using standardized methods (Coburn-Miller et al., 2015). Body mass index, an individual's weight in Kg divided by the square of their height ($BMI = kg/m^2$), was calculated for each participant and according to the WHO criteria (Edgar, 2013) and was used to classify participants' BMI as underweight < 18.5 , normal > 18.5 and < 24.9 , overweight > 25 and < 29.9 , obese > 30 and < 40 . The exclusion criteria included those who have had colon cancer or inflammatory bowel disease, experienced acute or chronic diarrhea in the 8 weeks prior to participation, had a course of antibiotics in the 2 months prior to participation and have been on food supplements or medications.

2.4. Blood sample collection

One milliliter of blood was collected in sterile tubes after overnight fast. The blood samples were then centrifuged (3500 rpm for 15 min) and the separated serum was transferred to appropriately labeled 1.5-mL Eppendorf tubes and stored at $-80^{\circ}C$ until use in experiments.

2.5. Multiplex chemokine assay

Multiplex chemokine analysis was performed on the serum collected from participants for MIG, monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein (MIP-1 α), MIP-1 β , regulated on activation, normal T cell expressed and secreted (RANTES), Eotaxin, interferon gamma-induced protein 10 (IP-10) and IL-8 using Multiplex immune-bead assay kit for human cytokines 30-plex panel (LHC6003M, Thermo-Fisher Scientific) according to the instructions given by the manufacturer. Briefly, antibody-coated polystyrene magnetic beads with different spectral intensities in solution were sonicated, then 25 μL was added to each well of the 96-well plate and washed twice with $1 \times$ wash buffer; this and subsequent washing steps were done using a handheld magnetic plate held at the bottom of the

96-well plate to retain the magnetic beads and prevent their loss. Standards (1:3 serial dilution) and samples (undiluted serum) were prepared, mixed with 100 μ L of the magnetic beads, and incubated at room temperature on an orbital shaker at 500 rpm for 2 h to enable capture of analytes. The wells were then incubated with 100 μ L biotinylated detection antibodies for 1 h, and the plate was subjected to two washes with buffer (200 μ L). Subsequently, streptavidin-RPE antibodies (100 μ L) were added for 30 min, and the plate was washed three times with wash buffer (200 μ L). The magnetic beads were then suspended in 100 μ L of wash buffer, and the data was acquired using a MAGPIX instrument (Luminex Corporation, USA). Finally, the chemokine expression data were analyzed using the Luminex xPONENT multiplex assay analysis software.

2.6. Statistical analyses

Statistical analysis was performed using the Statistical Package for Social Sciences version 24.0 for Windows (SPSS Inc., Chicago, IL, USA). Prism GraphPad version 6.0 (La Jolla CA, USA) was used. The Student's *t*-test was used to compare between groups and within groups, respectively. The data was expressed as mean \pm SEM (standard error of the mean). In addition, the Jonckheere – Terpstra test for ordered alternatives was carried out to investigate whether significant differences existed between the various chemokines tested in relation to BMI. Jonckheere – Terpstra test was used because the BMI levels were listed in an ordinal trend manner. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Multiplex chemokine assay – serum chemokines in men

The multiplex chemokine analyses indicated that the serum MIG levels were significantly higher in obese men ($P = 0.0194$) as

compared with the underweight men (Fig. 1). Similarly, IL-8, MIP-1 α , and MIP-1 β levels were increased in obese men compared with men in the underweight and overweight categories (Fig. 1). A nonsignificant increase in the levels of eotaxin was observed in normal, overweight, and obese men compared with underweight men. Furthermore, a nonsignificant increase was observed in the levels of IP-10 in obese men compared with other groups. However, serum levels of other chemokines, such as MCP-1 and RANTES, did not differ in underweight, overweight, and obese men compared to men with normal weight (Fig. 1).

3.2. Multiplex chemokine assay – serum chemokines in women

The multiplex chemokine analyses indicated that the serum MIG levels in obese women were significantly higher ($P = 0.043$) than in women in the underweight group (Fig. 2). Likewise, the overweight group had higher plasma MIG levels compared to the other two groups. However, the increase in the observed levels were not statistically significant. A nonsignificant increase was observed in the levels of MCP-1 and eotaxin in the obese group compared with the underweight and overweight groups (Fig. 2). In addition, a nonsignificant increase was observed in the plasma MIP-1 α levels in overweight females compared to other groups. Moreover, a nonsignificant increase was observed in the IP-10 levels in obese women compared with other groups. However, serum levels of other chemokines measured in the MAGPIX assay, such as IL-8, RANTES, and MIP-1 β , did not differ between the underweight, overweight, and obese groups compared to the normal group (Fig. 2).

3.3. Multiplex chemokine assay – serum chemokines combined (men and women)

The combined multiplex chemokine analyses in men and women indicated that the serum MIG levels in obese individuals

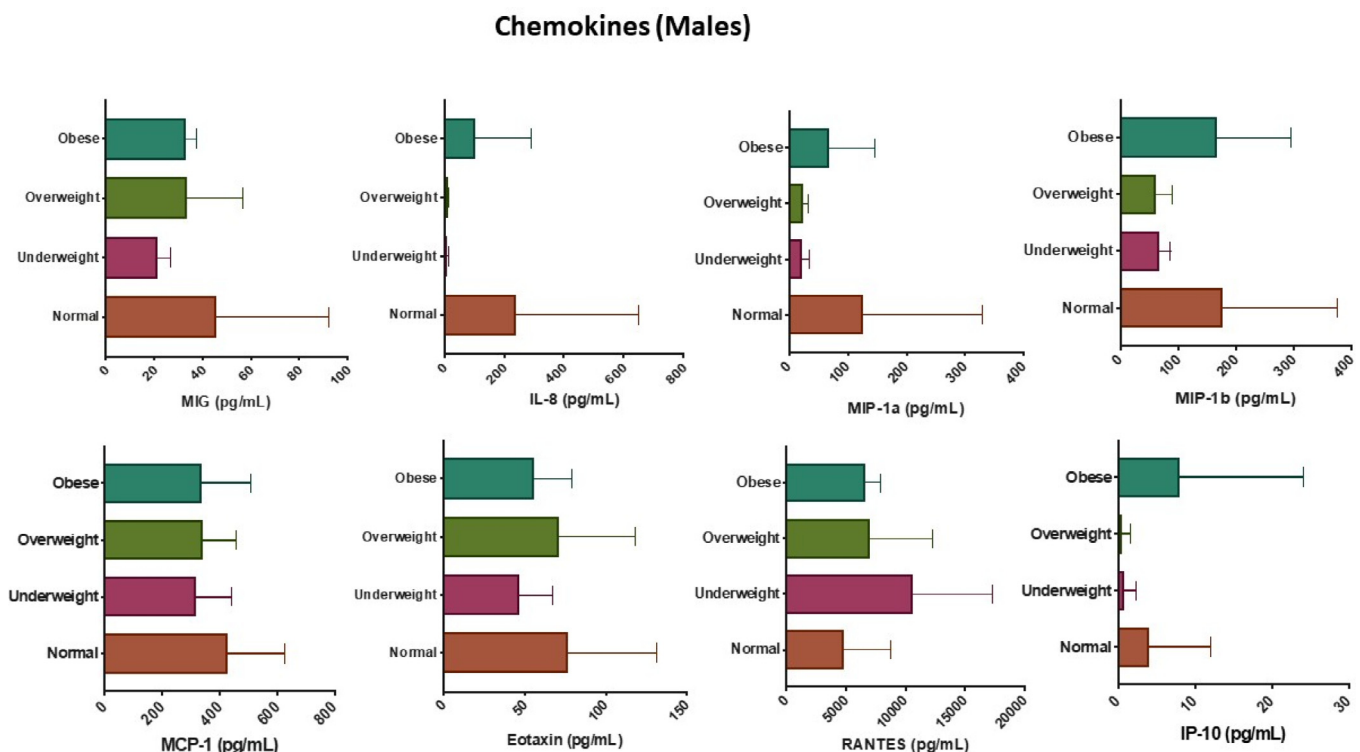


Fig. 1. Serum Chemokines in Men.

Chemokines (Females)

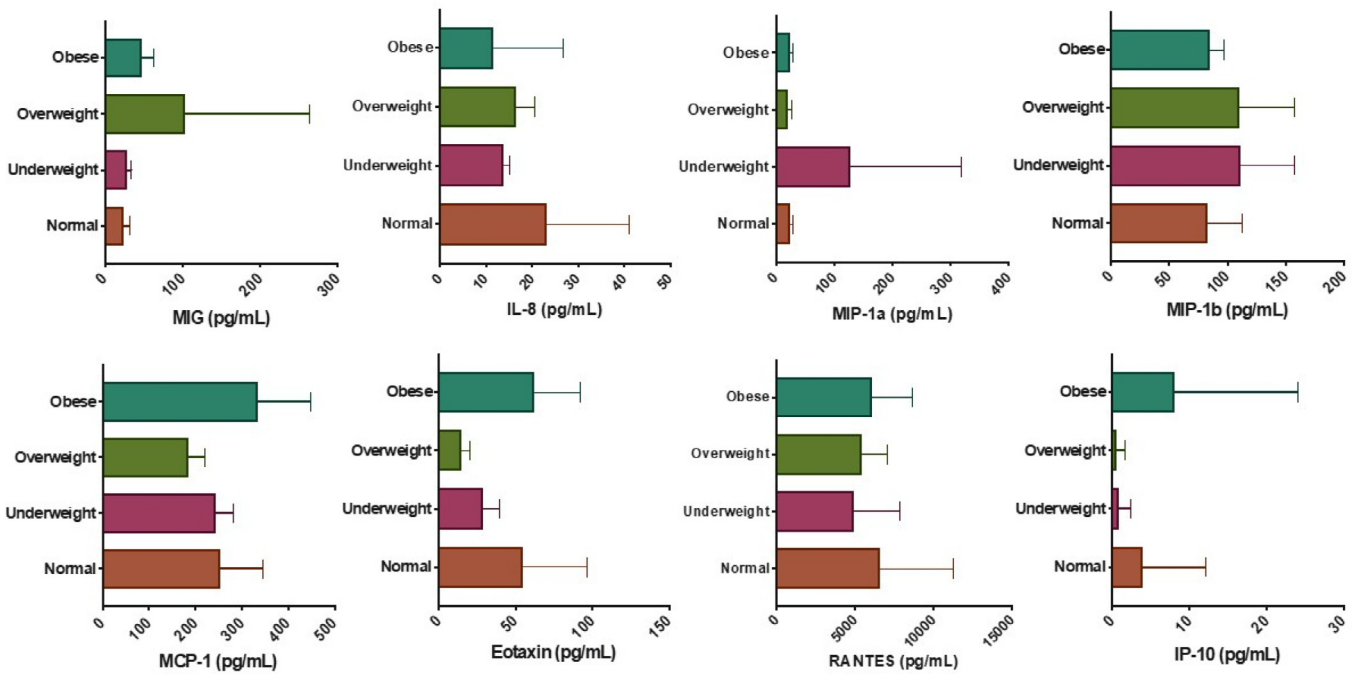


Fig. 2. Serum Chemokines in Women.

were significantly higher compared with the underweight group ($P = 0.0094$) (Fig. 3). Furthermore, eotaxin levels were increased and approached borderline significant in obese individuals compared to those who were underweight ($P = 0.069$) (Fig. 3). Similarly, IL-8 levels were borderline significantly increased in

overweight individuals compared with those in the underweight group ($P = 0.0922$). A nonsignificant increase was observed in the IP-10 levels in the obese group compared with other groups (Fig. 3). However, serum levels of other chemokines such as MCP-1, RANTES, MIP-1 α , and MIP-1 β , did not differ in the under-

Chemokines (Combined)

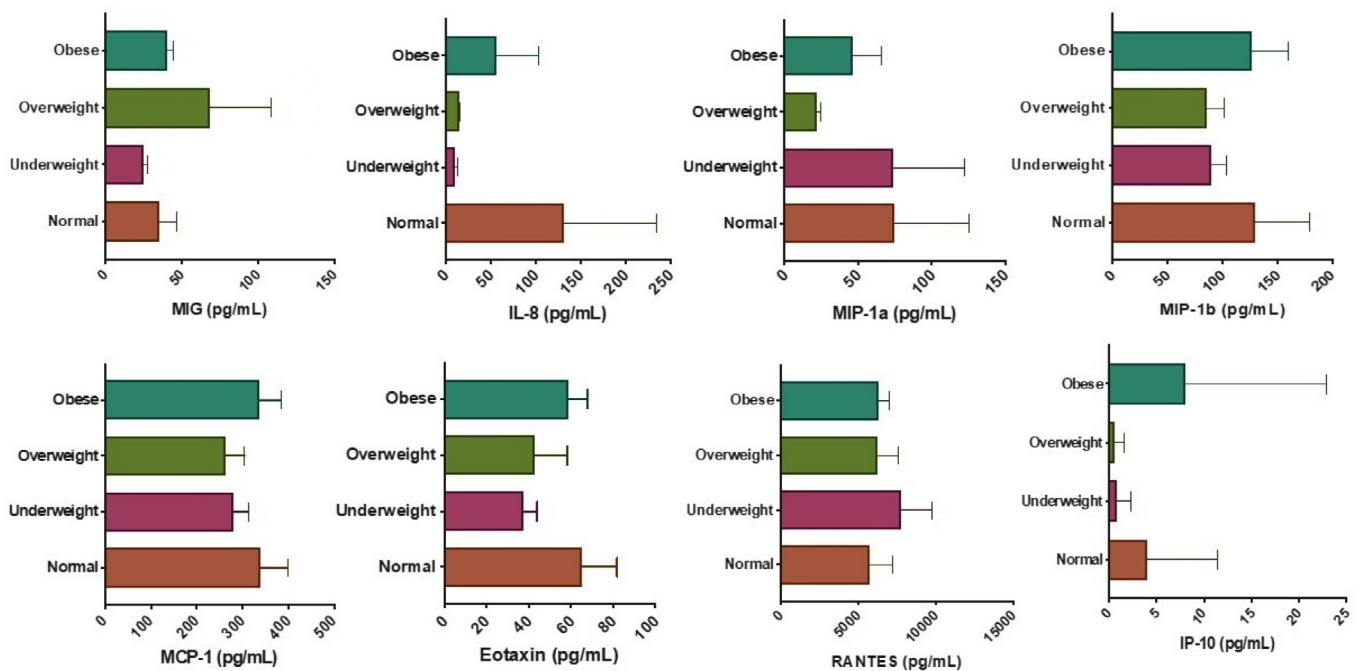


Fig. 3. Serum Chemokines Combined (Men and Women).

weight, overweight, and obese men compared to men in the normal group (Fig. 3). The multi-comparison test of Jonckheere – Terpstra test was also used since the BMI levels were listed in an ordinal trend manner. Based on the Jonckheere – Terpstra test, the results showed that there was a significant difference in the levels of MIG in relation to BMI in case of the underweight versus obese when including all the study participants.

4. Discussion

We have analyzed serum chemokines in underweight, overweight, and obese males and females and compared the levels with normal control individuals among Saudi citizens. Here, we showed that MIG levels are significantly increased in obese individuals with disregard to gender, and that eotaxin and IL-8 approached a significant increase in obese individuals. Systemic low-grade inflammation has been reported in overweight and obese individuals (Ota, 2013; Xu et al., 2015). This low-grade inflammation is chronic and potentially induces release or production of pro-inflammatory cytokines and chemokines, adipokines, and lipids in obese individuals, which could eventually lead to diabetes (Winer et al., 2009; Xu et al., 2015). The systemic chronic inflammatory state present in obesity is one of the underlying causes for the initiation, progression, and development of insulin resistance; it is also associated with co-morbid conditions such as type 2 diabetes mellitus, metabolic syndrome, nonalcoholic fatty liver disease, cardiovascular disease, and increased susceptibility to cancer (Xu et al., 2015; Yao L and Heuser-Baker J, 2014).

The plasma levels of MCP-1, also known as chemokine C-C motif ligand 2 (CCL2), is reported to be higher in obese children and adults compared to lean individuals (Breslin et al., 2012). In line with the above study, our results demonstrated an increase in the levels of MCP-1 in obese women compared to normal (Figs. 1 and 2). Although, MCP-1 was generally decreased in overweight and obese men compared to the control, the observed results were not statistically significant. The actual reason for the observed decrease in the trend remains obscure and needs further investigation. It is known that over nutrition triggers hypertrophy of adipocytes that secrete MCP-1, which in turn stimulates the migration of monocytes from the bloodstream into adipose tissues. There, monocytes mature into adipose tissue macrophages (ATMs) and promote inflammation through the production of pro-inflammatory cytokines and chemokines (Hotamisligil and Erbay, 2008; Xu et al., 2015). ATMs overexpress C-C motif chemokine receptor 2 (CCR2) and its ligand MCP-1, which are essential for the development of insulin resistance (Sartipy and Loskutoff, 2003), prediabetes, and hepatic steatosis in obesity (Kanda et al., 2006). In db/db mice, inhibition of MCP-1 improves insulin resistance and hepatic steatosis (Tamura et al., 2008). Essentially, MCP-1 modulates inflammation and metabolic effects in high-fat feeding (Weisberg et al., 2006).

Oral lipid tolerance test following overnight fasting in healthy volunteers was associated with increase in RANTES (CCL5) levels (Schmid et al., 2016). CCL5 acts through its receptor CCR5 along with MIP-1 α (CCL3), and MIP-1 β (CCL4) which are also implicated in obesity (Xue et al., 2018). Likewise, in the present study, RANTES were marginally increased in obese men compared to their normal counterparts and was comparable amongst obese and normal women (Figs. 1 and 2). Eotaxin levels are increased in obese individuals, and their levels have been found to be higher in a diet-induced obesity model (Clement et al., 2004; Vasudevan et al., 2006). However, we observed that eotaxin-1 levels were increased in obese women and near normal in obese men (Figs. 1 and 2). Chemokines are secreted in response to hypothalamic inflammation and in turn is associated with involuntary weight loss

(Le Thuc et al., 2017). Intracerebral-ventricular injection of two different families of chemokines, namely the CXC-motif chemokine ligand (CXCL) and the CCL2 in rats revealed that CXCL4, CXCL8, CXCL10, CCL2, and CCL5 cause decreased food intake (Plata-Salaman and Borkoski, 1994).

Levels of IL-8, MIG, and IP-10 have been clearly demonstrated to be increased in relation to obesity (Duarte et al., 2015; Laine et al., 2007; Zhang et al., 2016). Serum levels of IL-8/CXCL8 are significantly higher in subjects with obesity compared to healthy controls (Borges et al., 2018). In contrast, the levels of IL-8 in the present study were decreased in both obese men and women compared to normal, although they were not statistically significant (Figs. 1–). The levels of IP-10 was increased in both obese men and women in the present study (Figs. 1–3). High intensity interval exercises is known to decrease IL-8 and increase IP-10 levels (Dorneles et al., 2016). IL-8 belongs to the CXC chemokine subfamily secreted by an array of cells including inflammatory and endothelial cells (Duarte et al., 2015; Xu et al., 2015). The function of IL-8/CXCL8 is to organize the recruitment of neutrophils into the inflamed tissues (Xu et al., 2015; Zhang et al., 2016). The role of MIG/CXCL9 is still largely unknown and are reported to be increased in obesity. However, in the present study their levels were mostly decreased in both obese men and women (Figs. 1–3). The MIG/CXCL9 and IP-10/CXCL10 binds with the chemokine receptor, CXCR3, which is abundantly expressed in regulatory T-cells (Deiuliis et al., 2011), activated T cells and memory T-cells (Kintscher et al., 2008; Winer et al., 2009), natural killer cells (Wu et al., 2007), as well as monocytes and macrophages, and it plays a role in diet-induced obesity and insulin resistance (Deiuliis et al., 2014). Additionally, MIG/CXCL9 levels increase with obesity (Duarte et al., 2015) and are higher in the livers of patients with hepatic steatosis. The MIG/CXCL9-CXCR3 axis could be an important target for the amelioration of liver fibrosis related to obesity (Duarte et al., 2015; Xu et al., 2015).

5. Conclusion

In conclusion, the serum concentration of chemokine induced by gamma interferon (MIG) or CXCL-9 was significantly increased in obese men and women as compared with underweight men and women, respectively. However, the serum levels of other chemokines were not significantly different among the tested groups. We found that MIG levels are differentially regulated in serum, based on individuals' BMI.

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