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Inflammatory and Metabolic Profiling of Obese Adolescents: Insights into IL-6 and CXCL2/CXCR4 Axis

Doaa Dawood Salman [1], Maryam I. Salman [1] and Rawaa AlChalabi [2]*

[1] Department of Biology, College of Science, University of Anbar, Iraq.
[2] Department of Molecular and Medical Biotechnology, College of Biotechnology, Al-Nahrain University, Jadiriya, Baghdad, Iraq.
*Corresponding Author. rawaa.alchalabi@nahrainuniv.edu.iq

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Abstract

Overweight and obesity in adolescents is a severe global public health concern, characterized mainly by continuous low-grade inflammation and metabolic alterations. The current study aimed to analyze the lipid profile, anthropometric markers and specific chemokines to study the physiological and immunometabolism changes in this population. A cross-sectional study was performed involving 90 male adolescents aged 12–18 years, recruited and categorized into three groups based on BMI (WHO guidelines): Group 1 (Controls, BMI<25 n=40), Group 2 (Class I Obesity, BMI<34, n=32), and Group 3 (Class II Obesity, BMI ≥ 35 n=18). Waist-to-Hip Ratio (WHR) and concentrations of various lipid fractions, Interleukin-6 (IL-6), CXCL2, CXCR4, Serum levels of CXCL2 and CXCR4 were measured by ELISA, while Enzymatic method for estimating lipid profile .Statistical analysis showed that age did not differ between the groups; however, body mass index and waist-to-hip ratio were significantly higher in the obese groups compared to the control group, with significant differences(P<0.001), Furthermore, an abnormality in lipid levels was observed, represented by an increase in triglycerides (TG) from 74.13±8.13 mg/dL in healthy individuals (G1) to 135.19±29.04 in (G2) and 148.3±25.5 mg/dL in (G3), and LDL, from 113.68±10.70 to 111.9±8.29 in(G2,G3), respectively, While cholesterol (HDL-C) decreased significantly from 54.53±5.82 mg/dL in (G1) to 33.44±4.37 in (G2) and 30.78±5.00 mg/dL in (G3) at a significant level (P<0.001). The results showed a significant increase in serum interleukin-6 levels in obese individuals, with the highest increase in the third group (22.65 ± 5.009). CXCL2 expression also increased significantly, reaching 175.7 ± 5.103 in severe obesity, while CXCR4 levels decreased significantly in the second group (101.6 ± 18.91) and increased only slightly in extreme obesity (133.01 ± 10.68).In conclusion, obesity in adolescence causes extensive metabolic and immune disturbance and markers of inflammation and chemokines could be useful candidates as early predictors of monitoring immune and metabolic health

Keywords: Adolescent obesity, IL-6, CXCL2, CXCR4, Lipid profile.

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Introduction

Obesity has become a serious metabolic problem that is characterized by abnormal accumulation of adipose tissue. The situation in Iraq is not different, as recent international statistics indicate that the prevalence of obesity in children and adolescents has tripled since the 70s [(1)], and this increase puts them at a high risk of developing early onset metabolic diseases, including cardiovascular diseases and insulin resistance. Severity of obesity in clinical practice is mostly estimated by anthropometric parameters such as Body Mass Index (BMI) as well as by the lipid profile which shows metabolic imbalance induced by obesity. Therefore, the identification of precise clinical management plans in this population is strongly dependent on specific physiological and immune-related biomarkers, because of the underlying pathophysiology where enlarged adipocytes, along with recruited immune cells, release various pro-inflammatory signaling molecules [(2)] .[(3)]

At the Immunological level, the pathological evolution of adolescent obesity is related to chronic low-grade inflammation resulting from signaling in hypertrophic adipocytes. Consequently, immunometabolism plays an essential role in directing inflammatory cells within adipose tissue. Research has recorded increased levels of some immune markers, including IL-6, which contributes an essential role in activating the inflammatory response related with obesity [(4)],[(5)]

The study of the chemokine CXCL2 and the receptor CXCR4 was selected due to their major role in regulating the chemotaxis of immune cells. CXCL2 acts as an early distress signal, promotes the recruitment and migration of immune cells to the site of inflammation, which expedite the release of pro-inflammatory cytokines such as IL-6 and escalate lipid profile abnormalities. Furthermore, the receptor CXCR4 represents a regulatory associate; its downregulation leads to immune imbalance, accelerating metabolic syndrome and cardiovascular risks at a young age. [(6)], [(7)], [(8)] ,[(9)]

In light of these data, and given the Limited of clinical studies investigated this aspect in adolescents, this study aims to evaluate the anthropometric, lipid, and inflammatory characteristics of obese adolescent males by measuring serum levels of IL-6 and CXCL2/CXCR4, and to determine the nature of the statistical correlation between obesity severity and these biomarkers thereby contributing to the identification of predictive markers that help in early detection metabolic risks.

Materials and Methods

Study Design and Population

Aimed at addressing the objectives, a cross-sectional study was carried out on a sample of 90 male adolescents aged 12–18 years. Between December 2024 and April 2025, participants were enrolled at Fallujah General Hospital, Iraq. Data were collected Consecutively; the study

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population was separated according to three populations based on Body Mass Index (BMI) = weight (kg) Height2.:

Group 1 (G1): A control group of 40 healthy adolescents as a normal BMI.
Group 2 (G2): Obese adolescents are those that were classified as Obesity Class I (BMI<35 kg/m2)
Group 3 (G3): Informally classified as Obesity Class II (BMI≥35 kg/m2) adolescent OBESE (n=18). These classifications are totally consistent with current pediatric guidelines, where a BMI ≥ 35 kg/m² represents a critical threshold for acute metabolic risk in adolescents[10].

Exclusion Criteria

Rules were followed strictly to ensure integrity of information. Also, not included were those with chronic illness. These cardiopathic diseases included diabetes mellitus and autoimmune disease. Also, we

excluded adolescents with current acute infections and use of anti-inflammatory and immunosuppressive agents. Furthermore, smokers, those who follow restrictive dietary regimens, and those who engage in strenuous physical exercise were excluded due to the potential for these factors to negatively impact the study's indicators.

Sample Collection and Preparation

A blood sample from a participant's vein (about 5-6 mL) was taken in processing tubes. Before centrifugation at 1000 x g for 15 minutes, the samples were left to clot at room temperature. He remaining serum was distributed in microcentrifuge tubes and stored at – 20ºC until the results of the biochemical and immunological tests were known.

Immunological biomarker and lipid profiles

Blood lipid levels including total cholesterol, triglycerides, and HDL cholesterol—were determined using a commercial enzyme-colorimetric assay. To ensure maximum accuracy in assessing blood lipids, LDL cholesterol levels were determined by direct enzyme-colorimetric measurement rather than using mathematical equations.

The system that operates automatically underlines the high precision and extremely low pipetting variance of serum concentrations of IL-6, CXCL2 and CXCR4. To produce accurate calibration curves and ensure quality control, all standards and internal controls were run in duplicate during each run. performed using the ELISYS UNO automated ELISA analyzer (HUMAN Diagnostics, Germany) Following protocol streamlining, serum samples were later analysed. The IL-6 (Catalogue No: SL1001Hu_1) detection range is 3 pg/mL–200 pg/mL and the sensitivity is 0.8 pg/mL. The CXCL2 (Catalogue No: SL3045Hu) detection range is 3 pg/mL–200 pg/mL and the sensitivity is 0.5 pg/mL. Assay CXCR4 (Catalogue No: SL3788Hu) has a range of 15 pg/mL–300 pg/mL and a sensitivity of 1 pg/mL. Intra-assay and inter-assay coefficient of variation (CV) of the system was found to be strictly less than 10% and 12%, respectively.

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Statistical Analysis

Statistical software (SPSS) was used to perform analysis on the data. Quantitative variables were expressed as Mean±Standard Deviation. In keeping with the unique clinical objectives of this study, planned priori pairwise comparisons were conducted using the Independent Student's t-test. This targeted approach enabled us to evaluate the physiological and immunological alterations for each type of obesity (Class I & Class II) separately from health control. All graphs and charts were created by GraphPad Prism [GraphPad Software, USA] to display the differential between the groups. (p-value <0.05) was considered statistically significant for all statistical tests.

Results and Discussion

Anthropometric Characteristics

As shown in table 1, the demographic and clinical characteristics of the subjects were demonstrated. The study groups appeared to be homogeneous age distribution, with no statistically significant differences between the control group and the obesity subgroups (p>0.05). The body mass index (BMI) in the control group was significantly lower than in the second and third groups (P<0.001). The waist-to-hip ratio in the first group was lower compared to the other groups. The waist-to-hip ratio between the two obese subgroups did not differ significantly (P=0.105). Table 1, Fig 1.

Table 1: Baseline anthropometric and clinical characteristics of the study participants data expressed as Mean±SD (n=90); p<0.05 indicates statistical significance

Parameter	Means ±SD			P-value		
	G1 (n=40)	G2 (n=32)	G3 (n=18)	G1&G2	G1&G3	G2&G3
Age (year)	15.65±1.64	15.53±1.54	15.78±1.59	0.75	0.78	0.59
BMI (kg/m^2)	20.20±1.05	32.11±1.54	36.31±0.67	<0.001	<0.001	<0.001
WHR	0.81±0.01	0.97±0.02	0.98±0.01	<0.001	<0.001	0.105

Note: Data are presented as Mean ± SD. G1: Control group; G2: Obesity Class I (BMI<35 kg/m2); G3: Obesity Class II (BMI≥35 kg/m2)

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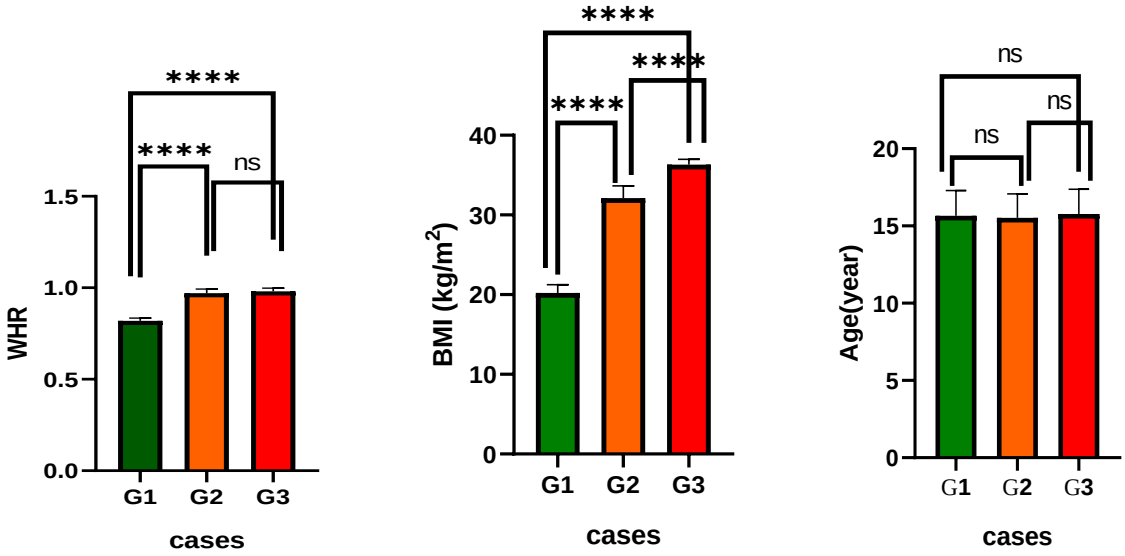

Figure 1 .Distribution of Age ,BMI and waist-to-hip ratio (WHR) across the study groups, Bars represent Mean±SD (n=90). symbols (*) indicate statistically significant pairwise differences at (p<0.05)

There was no difference in the mean age between study groups and hence no confounding effect of age in the physiological assessments. This limited age window (12–18 years) offers a framework in which to assess the changes in obesity during this sensitive period. The observations are like those made by [11], where they indicated no significant statistical differences of obesity parameters with age in adolescents.

Participants were divided into three groups for BMI progression, enabling a fine-grained look at how obesity affected participants. Our results confirm more recent regional studies [12], [13], that have reported a marked increase in overweight and obesity rates amongst adolescents.

population. While the Waist-to-Hip Ratio (WHR) was significantly higher in obese groups compared to controls, the lack of a significant difference between G2 and G3 suggests a "plateau effect" in central adiposity distribution at higher BMI levels. While WHR remains a hallmark of central obesity [12], our results support the perspective of [14], who argued that supplementary indices like the Fat Mass Index (FMI) may provide a more comprehensive assessment of metabolic risk.

lipid profile

The serum lipid profiles (TG, TC, LDL-C, and VLDL) were significantly higher in both obese groups as compared with the healthy controls (P<0.001; see Table 2 and Fig 2). In contrast, obese groups had significantly reduced levels of HDL-C (P<0.001).

The dysregulated lipid profile noted, with hypertriglyceridemia, high LDL-C and low HDL-C is similar to the 'atherogenic' phenotype often seen in obese adolescents [15] . These metabolic changes

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can be clinical markers of cardiovascular risk and the development of metabolic syndrome[16],[17]. Unfortunately, the marked Decreasing HDL-C is a worrisome one as it is important to reverse cholesterol transport and has anti-inflammatory properties, therefore if it is lost, it is a loss of critical vascular protection.[18][19]

Table 2: Difference in serum lipid profile of control and obese groups. data expressed as Mean±SD (n=90); p<0.05 indicates statistical significance

Parameter	Means ±SD			P-value		
	G1 (n=40)	G2 (n=32)	G3 (n=18)	G1&G2	G1&G3	G2&G3
TG (mg/dL)	74.13±8.13	135.19±29.04	148.3±25.50	<0.001	<0.001	0.114
TC (mg/dL)	153.78±7.65	186.25±11.01	186.9±23.10	<0.001	<0.001	0.88
LDL (mg/dL)	84.42±9.88	113.68±10.70	111.9±8.29	<0.001	<0.001	0.55
HDL (mg/dL)	54.53±5.82	33.44±4.37	30.78±5.00	<0.001	<0.001	0.056

Note: Data are presented as Mean ± SD. G1: Control group; G2: Obesity Class I (BMI<35 kg/m2); G3: Obesity Class II (BMI≥35 kg/m2), Significant difference at P<0.05.

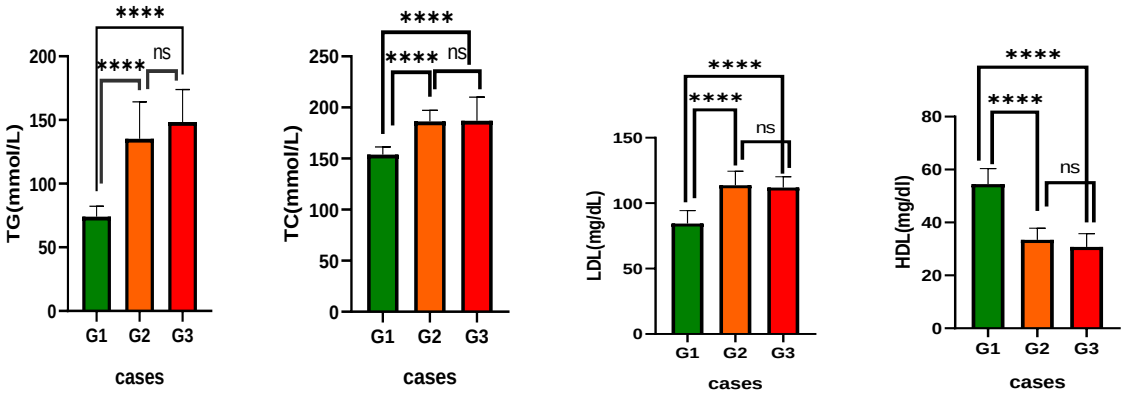

Figure 2. Comparison of blood fat levels in healthy and unhealthy groups. Bars represent Mean±SD (n=90). symbols (*) indicate statistically significant pairwise differences at (p<0.05)

Immunological biomarker

The immunological assay revealed that IL-6 levels were significantly higher in G2 and G3 than in G1 (P<0.001). A similar trend was observed in two groups of G3, which had the highest concentration.

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Interestingly, the expression of CXCR4 varied in a severity-dependent manner. In the G3 group, the level was significantly up-regulated as compared to controls (P<0.001). However, the difference between the G2 group and control group was nonsignificant as shown in Figure 3 and see Table 4.

Table 3: The relationship between immunological biomarker levels and obesity severity (IL-6, CXCL2, and CXCR4). data expressed as Mean±SD (n=90); p<0.05 indicates statistical significance

Parameter	Means ±SD			P-value		
	G1 (n=40)	G2 (n=32)	G3 (n=18)	G1&G2	G1&G3	G2&G3
CXCL2(pg/ml)	54.48±18.65	139±12.95	175.7±5.103	<0.001	<0.001	<0.001
CXCR4(pg/ml)	124.3±25.13	101.6±18.91	133.01±10.68	<0.001	0.07	<0.001
IL-6(pg/ml)	6.12±1.75	16.31±4.69	22.65±5.009	<0.001	<0.001	<0.001

Note: Data are presented as Mean ± SD. G1: Control group; G2: Obesity Class I (BMI<35 kg/m2); G3: Obesity Class II (BMI≥35 kg/m2), Significant difference at P<0.05

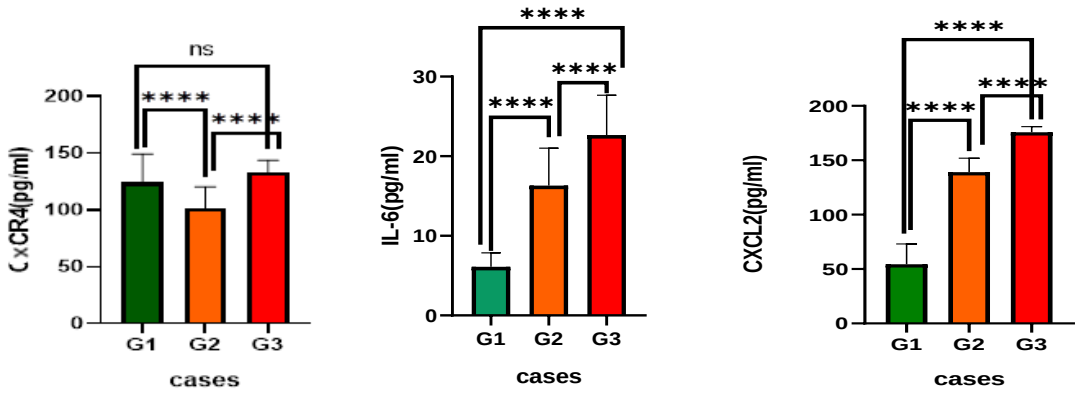

Figure 3. Serum concentrations of IL-6, CXCL2, and CXCR4 across the study cohorts, Bars represent Mean±SD (n=90). symbols (*) indicate statistically significant pairwise differences at (p<0.05)

The increased serum levels of IL-6 in the obese groups supports IL-6 as a principal mediator which contributes to obesity-related chronic inflammation. This is in keeping with a number of recent studies[20][21]. Mechanistically, IL-6 has been shown to be involved in the

promotion of visceral fat deposition and the suppression of lipolysis, through its suppression of HSL and ATGL activities. While some studies in adults reported lower[22], These discrepancies are probably due to age and sex differences in cytokine dynamics [23]

One of the important findings of this study is the dysregulated expression of the chemokines CXCL2 and CXCR4. CXCL2, an important factor in the recruitment process of neutrophils, increased significantly, especially in group (G3). This is consistent with the [24] work, which indicates that CXCL2 plays a direct role in the development of inflammatory complications of advanced obesity[25].

In the case of CXCR4, our results showed that it was expressed differently depending on the severity. It was significantly increased in the severe obesity group (G3) but did not demonstrate a statistically significant difference in the Class I obesity group (G2) versus controls. This "threshold effect" suggests that the ability of CXCR4 to promote leukocyte migration into adipose tissue begins to be a predominant pathological trait at more advanced stages of obesity [26]. Together, the synergistic increase of CXCL2 and CXCR4 suggests a hypothesis that metabolic disorders associated with obesity are related to chemokine signaling dysfunction which leads to systemic inflammation and immune cell infiltration [27],[28]

Study Limitations

This study has some limitations, including a male-only sample, which limits the generalizability of the results to females. This methodology was deliberately adopted to exclude a specific sex and avoid the potential influence of hormonal changes during puberty, which are known to significantly affect immune and metabolic markers and inflammatory responses. Therefore, future longitudinal studies involving both sexes are needed to account for potential sex differences in these pathways.

Conclusion

The results of this study showed an association between male adolescent obesity and lipid, anthropometric, and immunometabolism changes characterized by a chronic inflammatory state that increases with the severity of obesity. The study also highlighted the potential role of the CXCL2/CXCR4 axis in promoting inflammation, given its marked elevation in morbid obesity. Early prediction of metabolic, cardiovascular, and circulatory risks can be made by utilizing these relevant indicators alongside anthropometric measurements and lipid profiles. Future studies should include longitudinal studies that follow these biomarkers over time, confirm the validity of these results in adolescent females, and explore whether long-term health complications of obesity can be reduced through lifestyle modifications and interventions that effectively modulate the CXCL2/CXCR4 axis.

Acknowledgment

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Author's Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine/ours. Furthermore, any Figures and images, that are not mine/ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- Ethical Clearance: was obtained from the Research Committee at the Training and Human Development Center, Anbar Health Directorate, Ministry of Health–Iraq (Approval No. 2025009; Date: 18 February 2025).

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