

Obesity and immune cells in Saudi females

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Innate Immunity
17(5) 439–450
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sagepub.co.uk/journalsPermissions.nav
DOI: 10.1177/1753425910372536
ini.sagepub.com



Abstract

For the past two decades or more, obesity has been increasing in industrialized and affluent countries. This increase has been shown in children, adults and the elderly. The latest statistical studies in Saudi Arabia show that obesity is increasing, with obese Saudi women outnumbering obese Saudi males, and that these numbers are continuously rising. Obesity, which leads to many medical risks, affects the immune system in direct and indirect ways. In this study, the effect of obesity on the immune system of 119 Saudi female university students was investigated. Using a blood sample from each subject, the following immune-related parameters were determined: total and differential white blood cell counts (WBCs), total lymphocyte and sub-lymphocyte cell counts. Two measures of body fat were used: the body mass index (BMI) and the waist-to-hip ratio (WHR) to correlate body weight to the other measured parameters. A significant correlation was present between BMI and WHR each with total white blood cells, neutrophils, and CD4 lymphocyte concentrations. Some other parameters were only affected by the increase in the BMI but not the WHR, such as platelets, while others were affected by the WHR only, such as total T-cells. On the other hand, there were no significant correlations between BMI or WHR and basophil, eosinophil, monocyte, CD8, B-cell, and NK-cell counts. The findings indicate that obesity might seriously affect the innate and adaptive immune systems.

Keywords

Obesity, immune cells, body mass index (BMI), waist-to-hip ratio (WHR), white blood cells (WBCs)

Date received: 16 January 2010; revised: 17 March 2010; accepted: 21 April 2010

Introduction

Obesity has been on the rise in many industrialized and affluent countries for the last 20 years or more. Data on the Saudi Arabian population confirm that obesity and overweight are increasing, and that obesity is higher in females than in males, with foreseen rises in the levels of several associated diseases and conditions.^{1–4} Obesity is a known major risk factor for many diseases (such as cardiovascular diseases, tumors, and diabetes) and may lead to a shortened life-span. It has also been shown that the obese have a higher non-specific inflammatory response.⁵ Due to the increasing prevalence of obesity and overweight and its associated risks, many scientists are studying the relationship between body weight and general health. The effect of overweight and obesity on immunity is one relationship that researchers are attempting to elucidate. Many studies on humans and animals have inconclusively shown that obesity affects the levels of immune cells and that there is an association between obesity and cellular immunity.⁶

There are several ways to judge body weight depending on different parameters, and each method has its

advantages and disadvantages. The two most common and accurate methods are body mass index (BMI), and waist-to-hip ratio (WHR). Body mass index reflects a person's weight in relation to height and thus gives a general indication of the healthy weight for height.⁷ The waist-to-hip ratio classifies subjects into three major risk groups for health problems.⁸ The WHR is the best indicator of central obesity, which increases the risk of cardiovascular disease.

Both innate and acquired immune responses are activated not only when a pathogen is present, but also in the case of abnormal conditions and malfunctions in the body, such as in the case of obesity. It has been shown that body weight affects the immune system in direct and indirect ways. One way to measure this effect

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on the immune system is to measure the concentrations of the different cells involved in immune responses.

Platelets play a major role in immunology, especially in inflammatory response.⁹ They contain granules with contents that result in leukocyte attraction and an increase in inflammation. It has been shown^{10–15} that platelet counts and activity are effected by body weight, obesity and WHR. This affect of obesity on platelet counts, in turn, interrupts and affects immunological functions. Platelet counts have been shown to rise in obese women and with increasing WHR.^{10,11,13}

Platelet hyperactivity is associated with atherosclerosis and cardiovascular disease.^{10,11} Contrary to the findings discussed above, inhibition of platelet activity has been found to be associated with obesity.¹² Studies^{14,15} on the effects of obesity on platelet counts found that the obese and high WHR groups had higher platelet activation. Systemic inflammation may be related to platelet activation by the inflammatory cytokines released in the process. Thus, this relation between platelet activation, inflammation and obesity is a link between the immune system and cardiovascular disease.

A study by Nieman *et al.*¹⁶ on a community of obese and non-obese women showed a general increase in leukocytes, monocytes, granulocytes, and neutrophil phagocytosis in obese women. Another study on obese adolescent females found a positive correlation between neutrophils and total white blood cell counts with BMI, while basophil counts were negatively correlated with BMI.¹⁷ An increase in the white blood cell counts is known to be a major risk factor for inflammatory responses,¹⁸ and coronary heart disease.¹⁹

Researchers showed that the leukocyte and lymphocyte subset counts are increased by obesity, except for natural killer (NK) cells and T cytotoxic/suppressor (CD8) cells.²⁰ Obesity also decreases the cell activity of NK cells,⁶ and the proliferation of mitogen-induced B- and T-cells.²⁰ A major role in the regulation of the immune response is played by NK cells, which are important in innate defense mechanisms and may be considered as a bridge which links innate and acquired immunities.²¹

A research study on females found that higher CD4, CD8, WBCs, and total lymphocyte counts were associated with overweight and obesity, while a higher CD8 count was associated with morbid obesity. In addition, no significant relationship was found between percentages of CD4 and CD8 or CD4/CD8 ratio and obesity.²²

In animal studies, obesity was found to affect cell-mediated immune response. It also leads to the enhancement of NK-cell activity. The number of functional T-cells decreased in the obese animals, which resulted in impaired immunity.⁶ These animal studies confirm the destructive effects of obesity on the immune system. In another study,²³ obese mice showed a higher sensitivity of antigen-induced T-cell responses.

The aim of this study was to determine the effects of overweight and obesity on cells of the immune system in healthy young females. This is important to better understand the relationship between increasing weight and the immune response in healthy individuals, especially since there are no data on this relationship in the local community. This may help set better guidelines for the ideal weight for a strong immune response.

Subjects and methods

Subjects and categories

A total of 119 randomly chosen Saudi female students (17–26 years old) at King Abdulaziz University (Jeddah, Saudi Arabia) took part in this study. The subjects filled a consent form and a questionnaire concerning their general health state and life style. Subjects with any health problems (such as diabetes and blood abnormalities) or taking any medications (such as anti-inflammatories or hormonal drugs, other than birth control pills) were excluded. Also, none of the subjects were menstruating or pregnant when blood samples were obtained.

Subjects were divided into five groups, or categories, based on their BMI. The underweight group included all subjects with a BMI below 18.9 kg/m²; the average or healthy weight group had a BMI of 18.9–24.9 kg/m²; the overweight group were those with a BMI between 25–29.9 kg/m²; obese subjects had a BMI range between 30–40 kg/m²; and, finally, a BMI above 40 kg/m² was defined as the highly obese category.

Using the WHR, subjects were also classified into one of three major risk groups. The cut-off points differ for females and males and thus the ones used here are specific for females. Subjects at a low risk for health problems are those with a WHR of 0.8 and below, moderate risk WHR is between 0.81–0.85, while high risk is a ratio above 0.85.⁸

Blood collection

Whole blood was collected, from each subject, into EDTA Vacutainer tubes, which were used within 3 h for differential complete blood counts (CBCs). Whole blood was collected into sodium heparin (NH) Vacutainer tubes for the determination of lymphocyte subsets since this allows the blood to be used for up to 3 days.

Concentration of white blood cells and differential complete blood count

Both the total white blood cells and differential CBCs were assayed at the King Abdulaziz University Hospital using a Coulter Counter (LH755; Miami, FL, USA). The total and differential (total

lymphocytes, monocytes, neutrophils, eosinophils, and basophils) white blood cell counts were determined using both fluorescence and optical scattering. Also, the platelet counts were determined.

Lymphocyte subset count

The lymphocyte subsets were determined in whole blood using a flow cytometer (FACSCanto II; Becton Dickinson, San Jose, CA, USA) at the King Khalid National Guard Hospital, Jeddah, Saudi Arabia.

To determine the counts of each lymphocyte class and subclass, different class or subclass specific antibodies (all from Becton Dickinson) were used. To determine the total concentration of lymphocytes, peridinin chlorophyll protein (PerCP)-labeled mouse anti-human-CD45 monoclonal antibody was used. The total percentage of T-lymphocytes was determined using a fluorescein-isothiocyanate (FITC)-conjugated mouse anti-human CD3 (FITC-CD3) monoclonal antibody (MAb). For the determination of CD4 cells, the above FITC-CD3 antibody was used in addition to a phycoerythrin (PE)-labeled mouse anti-human CD4 monoclonal antibody. For the determination of CD8 cells, the above mentioned FITC-CD3 was used in addition to a PE-labeled mouse anti-human CD8 monoclonal antibody. A PE-labeled mouse anti-human CD19 monoclonal antibody was used for the determination of B-lymphocytes. PE-labeled mouse anti-human CD16 and CD56 monoclonal antibodies were used for the determination of the concentrations of NK-cells in whole blood.

Staining method

In vitro diagnostic use Becton Dickinson reagents were used for the determination of the major lymphocyte subsets by using three color lymphocyte immunophenotyping. Whole blood (100 µl) was added to the lineage-specific antibodies (as described above) in each tube. After being gently vortexed, the tubes were incubated in the dark for 20 min, to ensure the binding between the antibodies and the cells. A Becton Dickinson FACS lysing solution was added to lyse the red blood cells (RBCs) and the solution was allowed to incubate in the dark for 10 min. Tubes were centrifuged for 3 min at 3000 rpm, the supernatant was decanted and the sample was washed using phosphate-buffered saline (PBS) solution. Subsequently, the tubes were again centrifuged for 3 min at 3000 rpm and the supernatant was decanted. Finally, a Becton Dickinson Cell Fixative solution was added to each tube.

The specimens were run on the above mentioned FACSCanto II flow cytometer. Before running samples, fluidic start-up, threshold adjustment, and voltage setting were performed. These are done to ensure the proper functioning of the instrument and the proper

acquisition of data. The immune complexes which resulted from the binding of the cells and the antibodies, in the previous step, deflect the laser light. The degree of scattering depends on the physical properties of the cell, mainly its size and internal complexity.

Gating lymphocytes

The correlation of CD45 and side-scatter (SSC) allows the differentiation of cell types by knowing the general area on the dot plot for lymphocytes, monocytes and granulocytes. The percentage of each cell type was obtained using the instrument's software. As confirmation, the sum of the percentages of B-, T-, and NK-cells in each gate should be $100 \pm 5\%$.

Statistical analysis

The statistical program SPSS v12 was used to obtain the descriptive and analytical statistics. Mean, SD, SE, and range (minimum–maximum) were calculated for each parameter.

After testing the normal distribution and the homogeneity of the populations, it was found that although the sample (all subjects) follows the normal distribution, since it is a large sample (exceeds 50), the data for some of the parameters are not homogeneous. So, the ANOVA one-way test was used to test the significance in the correlation between the BMI or WHR and each parameter measured for each subject. After using the ANOVA test, the post hoc tests were used for multi-comparisons. The Tukey test was used for the homogeneous parameters, while Tamhane's T2 test was used for the non-homogeneous parameters. The resulting *P*-values demonstrate significance or lack thereof. The cut-off points used were: $P > 0.05$ is a non-significant difference (NS), $P < 0.05$ is a significant difference (S), and $P < 0.01$ is a highly significant difference (HS).

Results

Subjects, BMI and WHR categories

The 119 female subjects used in this study had an age range of 17–26 years (mean \pm SD, 21.68 ± 1.895 years), and a BMI range of 13.77 – 55.26 kg/m² (mean \pm SD, 28.2480 ± 9.55157 kg/m²). As evident in Figure 1, the age is normally distributed since the data are scattered about the line.

At a confidence level of 99%, it is noted that the mean of the BMI belongs to the confidence interval (26.4513, 30.0447) which means a fairly narrow range of BMI values as appears from the SD (9.55157), so the SE (0.90659) is very small. Subjects were chosen within the specific age interval 17–26 years based on the BMI strata (Fig. 2). That is, each stratum subject was randomly selected so that there are more than 20 subjects per stratum.

The subjects were divided into five groups according to their BMI, as shown in Table 1. Each group had 21–26 subjects. The five groups are: underweight subjects (frequency, percentage: 24, 20.2%), healthy subjects (26, 21.8%), overweight subjects (23, 19.3%), obese subjects (25, 21.0%), and highly obese subjects (21, 17.6%).

As shown in Table 1, the minimum BMI for the underweight category is 13.77 kg/m², while the maximum (18.73 kg/m²) is nearly equal to the accepted maximum for the underweight category of 18.9 kg/m². For healthy subjects, who are considered the control group, the range of BMI for the subjects was 19.07–24.39 kg/

m², nearly encompassing the whole range (18.9–24.9 kg/m²) specified for the healthy category. Also for the overweight subjects, the BMI range is 25.31–29.76 kg/m², which compares well with the accepted range of 25–29.9 kg/m². The obese subjects range from 30.02–39.41 kg/m², compared with the accepted range of 30–40 kg/m². Finally, the highly obese start with a BMI of 40.08 kg/m² and reach a maximum of 55.26 kg/m², which compares with the accepted BMI of above 40 kg/m² for the highly obese.

The subjects have WHRs that range from 0.2–1.62 (mean ± SD, 0.7659 ± 0.13830) with a very small SE of 0.01313 and a short confidence interval (0.68, 0.7226), which shows a fairly narrow range of WHR values. The WHRs were divided into three groups as shown in Table 2. The three groups are: subjects with low risk (frequency, percentage: 82, 68.9%), subjects with moderate risk (11, 9.2%), and subjects with high risk (26, 21.8%).

Body mass index categories and immune system cells

The mean values of serum platelet counts (Table 3) increased as the BMI increased, reaching its maximum value in the obese group, while it decreased in the underweight group. The ANOVA one-way test showed that these differences are highly significant for all groups.

Surprisingly, the Tukey test did not show any significant differences between the healthy group and other groups; therefore, the LSD test was used as a post hoc test (Table 4), which showed that the increase in the

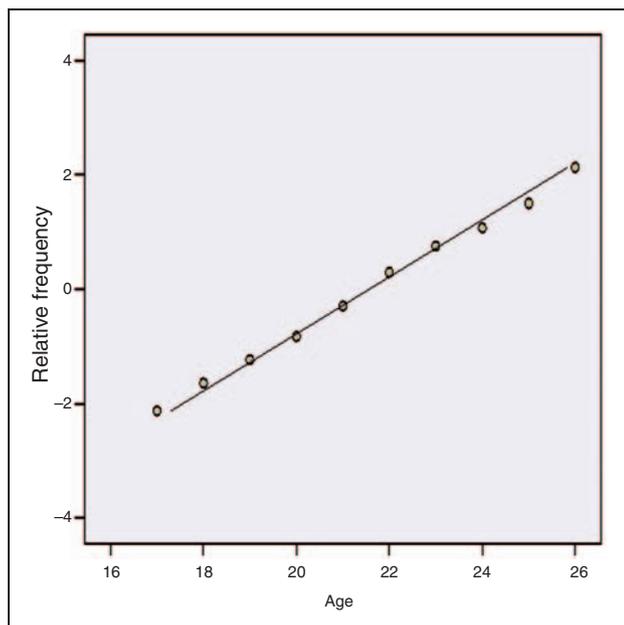


Figure 1. Normal distribution test of the subjects' ages.

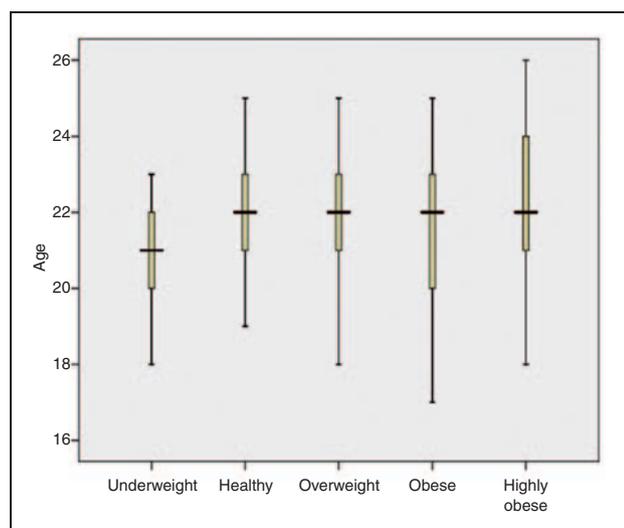


Figure 2. Box-plot of BMI strata versus age. The middle horizontal line is the mean for the respective group.

Table 1. Frequency and calculated percentages of subjects in the five BMI categories

BMI	Frequency	Percentage	Minimum BMI (kg/m ²)	Maximum BMI (kg/m ²)
Underweight	24	20.2%	13.77	18.73
Healthy	26	21.8%	19.07	24.39
Overweight	23	19.3%	25.31	29.76
Obese	25	21.0%	30.02	39.41
Highly obese	21	17.6%	40.08	55.26
Total	119	100%		

Table 2. Frequencies and calculated percentages of subjects in the five WHR categories

WHR	Frequency	Percentage
Low risk	82	68.9%
Moderate risk	11	9.2%
High risk	26	21.8%
Total	119	100%

platelet counts in both the overweight and highly obese subjects is significant in comparison with the healthy group. Also, a highly significant increase in the platelet counts was obtained for obese subjects compared to the healthy group (Table 4).

Statistical analysis (Table 3) showed the effect of BMI on the total white blood cell counts. Using the ANOVA one-way test, the mean values of the total white blood cell counts showed a highly significant increase as the BMI increases when compared to the healthy (control) BMI group. This increase in the mean values is statistically highly significant only in the obese and highly obese groups in comparison to the healthy BMI group, using the multiple comparisons test between groups (Table 5).

Table 3 shows that the mean values of the basophil cell counts for all groups were not significantly different

from the healthy group. Thus, the ANOVA one-way test showed that there was no significant correlation between the BMI and the counts of basophils. The differences for the eosinophil and monocyte cell counts compared individually to the healthy group (Table 3) were statistically non-significant.

Finally, Table 3 shows the effect of the BMI on neutrophil cell counts. All categories give higher counts of neutrophil cells compared with the healthy group. The mean reached its maximum value in the obese group. The ANOVA one-way test showed that the correlation between BMI and neutrophil white blood cell counts were highly significant. The Tukey post hoc test was used for multiple comparisons (Table 5) and showed a highly significant increase of the neutrophil white blood cell counts in both the obese and highly obese groups compared to the healthy group.

Table 3. Descriptive statistics and test of significance for WBC, platelet, basophil, eosinophil, monocyte, and neutrophil cells counts for the BMI categories

	BMI	Max	Min	Mean	SE	SD	P-value
Platelet counts	Underweight	509	151	289.2609	17.9316	85.99694	0.009 ^{HS}
	Healthy	506	217	291.6923	12.70588	64.78751	
	Overweight	477	212	339.55	16.63248	74.38271	
	Obese	480	275	347.3810	12.39306	56.79214	
	Highly obese	465	183	334.2381	14.29784	65.52092	
WBC counts	Underweight	9.73	4.39	6.5591	0.27462	1.31704	0.000 ^{HS}
	Healthy	9.4	3.1	5.9462	0.31478	1.60505	
	Overweight	10.6	3	6.8350	0.41575	1.85927	
	Obese	12.4	5.4	8.2762	0.43211	1.98017	
	Highly obese	12	5.25	8.4176	0.43744	2.00459	
Basophil counts	Underweight	0.10	0.00	0.0261	0.00936	0.04490	0.474 ^{NS}
	Healthy	0.10	0.00	0.0320	0.00952	0.04761	
	Overweight	0.40	0.00	0.0524	0.02026	0.09284	
	Obese	0.10	0.00	0.0500	0.01091	0.05118	
	Highly obese	0.10	0.00	0.0476	0.01117	0.05118	
Eosinophil counts	Underweight	0.20	0.00	0.1043	0.01472	0.07057	0.077 ^{NS}
	Healthy	0.60	0.00	0.1462	0.02895	0.14760	
	Overweight	0.50	0.00	0.1350	0.02927	0.13089	
	Obese	0.70	0.00	0.1381	0.03198	0.14655	
	Highly obese	1.10	0.00	0.2333	0.04992	0.22876	
Monocyte counts	Underweight	0.70	0.30	0.4870	0.02539	0.12175	0.900 ^{NS}
	Healthy	0.80	0.20	0.5115	0.02900	0.14786	
	Overweight	0.80	0.20	0.4800	0.04329	0.19358	
	Obese	0.70	0.30	0.5048	0.02966	0.13593	
	Highly obese	0.80	0.30	0.5190	0.03057	0.14007	
Neutrophil counts	Underweight	1.40	4.70	3.1304	0.20875	1.00111	0.000 ^{HS}
	Healthy	1.30	6.00	2.9500	0.25043	1.27695	
	Overweight	1.10	7.50	3.6350	0.35860	1.60370	
	Obese	2.40	7.90	4.7476	0.34056	1.56065	
	Highly obese	2.00	7.10	4.5952	0.33880	1.55257	

HS, highly significant ($P < 0.01$)

NS, not significant.

Table 4. Multiple comparisons between mean platelet counts for the healthy group and other groups

	BMI (H)	BMI (Cat)	Mean differences		P-value Tukey	LSD
			(H-Cat)	SE		
Platelets	Healthy	Underweight	2.43144	20.10933	1.000	0.904
		Overweight	-47.85769	20.89428	0.156	0.024 ^S
		Obese	-55.68864	20.61117	0.060	0.008 ^{HS}
		Highly obese	-42.54579	20.61117	0.243	0.041 ^S

The mean difference is significant against healthy weight subjects at the 0.05 level.

HS, highly significant ($P < 0.01$); S, significant ($P < 0.05$).

H, healthy; Cat, category.

Table 5. Multiple comparisons between the mean WBC and neutrophil counts for the healthy group and other groups

	BMI (H)	BMI (Cat)		Mean differences		P-value
				(H-Cat)	SE	
WBC counts	Healthy	Underweight	Tukey	-0.61298	0.50280	0.740
		Overweight		-0.88885	0.52242	0.438
		Obese		-2.33004	0.51534	0.000 ^{HS}
		Highly obese		-2.47147	0.51534	0.000 ^{HS}
Neutrophil counts	Healthy	Underweight	Tukey	-0.18043	0.40156	0.991
		Overweight		-0.68500	0.41724	0.474
		Obese		-1.79762	0.41158	0.000 ^{HS}
		Highly obese		-1.64524	0.41158	0.001 ^{HS}

The mean difference is significant against healthy weight subjects at the 0.05 level.

HS, highly significant ($P < 0.01$).

H, healthy; Cat, category.

The ANOVA one-way test showed (Table 6) a highly significant correlation between BMI and lymphocyte cell counts. The Tukey post hoc test showed (Table 8) a significant increase in the lymphocyte cell counts in the highly obese over the healthy group, while other groups showed no significant difference. The mean values of the T-, CD8-, B- and NK-cell counts and the mean values of the CD4 T-cell percentages, CD8 T-cell percentages, CD19 B-cells, CD56 + CD16 NK-cells, and CD4/CD8 ratio for all categories are shown in Tables 6 and 7. The ANOVA one-way test shows no significant relationships between these absolute cell counts, percentages and ratio with the BMI.

The ANOVA one-way test, which was used to test the significance of the correlation between CD4 cell counts and BMI, showed a significant relationship between the CD4 cell absolute counts and the BMI. The Tukey post hoc test showed a significant increase in the CD4 cell counts only in the highly obese group as compared to the healthy group (Table 8).

Waist-to-hip ratio categories and immune system cells

The mean values for the platelet, basophil, eosinophil, and monocyte cells counts (Table 9) were determined.

The mean values are not significantly different for both the moderate and high-risk groups when compared to the low-risk group (control).

Table 9 shows the mean values of the total white blood cells in the blood for the WHR categories. The maximum and minimum values are also reported for each group. The ANOVA one-way test showed a highly significant relationship between total WBCs and WHR. The Tukey post hoc test was used for the multiple comparisons; it was found that the difference between the mean values of the low-risk group (control) and the other groups are highly significant, as shown in Table 10.

The mean values for blood neutrophil cell counts for both the moderate and high-risk groups (Table 9) showed a highly significant increase compared to the low-risk group. The highest mean neutrophil cell counts are for the moderate risk group (Table 9). The Tukey post hoc multiple comparisons test (Table 10) was used to compare the neutrophil mean values of the WHR groups; there was a highly significant difference in the results for the three risk groups.

No significant effect of the WHR on the lymphocyte cell counts was found (Table 11); nevertheless, the P -value was only slightly higher than the significant value (only 0.002 higher). Therefore, the difference would become significant if a post hoc test, such as LSD, were used.

Table 6. Descriptive statistics and test of significance for lymphocytes, and lymphocyte subsets cells counts for the BMI categories

	BMI	Max	Min	Mean	SE	SD	P-value
Lymphocyte cell counts	Underweight	6.00	1.70	2.7957	0.19454	0.93297	0.012 ^{HS}
	Healthy	4.10	1.30	2.2923	0.11857	0.60460	
	Overweight	4.40	1.40	2.5100	0.15810	0.70703	
	Obese	4.80	1.70	2.8619	0.16410	0.75198	
	Highly obese	5.60	1.80	3.0238	0.17727	0.81235	
T lymphocyte cell counts	Underweight	4.82	1.17	2.1544	0.1663	0.79963	0.55 ^{NS}
	Healthy	3.15	0.88	1.7508	0.09395	0.47903	
	Overweight	3.12	1.15	1.9138	0.11694	0.52297	
	Obese	4.13	0.00	2.1636	0.17350	0.81380	
	Highly obese	4.18	1.35	2.2660	0.13761	0.63063	
CD4 cell counts	Underweight	2.53	0.48	1.1994	0.09642	0.46240	0.03 ^S
	Healthy	1.80	0.56	1.0154	0.05641	0.28761	
	Overweight	1.94	0.62	1.1327	0.07205	0.32223	
	Obese	2.35	0.00	1.2018	0.09703	0.45512	
	Highly obese	2.56	0.74	1.3981	0.09569	0.43850	
CD8 cell counts	Underweight	2.17	0.44	0.8294	0.07927	0.38015	0.076 ^{NS}
	Healthy	1.20	0.29	0.6413	0.04010	0.20449	
	Overweight	1.23	0.35	0.6802	0.05602	0.25052	
	Obese	1.53	0.00	0.8419	0.08153	0.38243	
	Highly obese	1.50	0.41	0.8165	0.06085	0.27883	
B cell counts	Underweight	0.78	0.16	0.3156	0.03160	0.15156	0.057 ^{NS}
	Healthy	0.79	0.10	0.3055	0.03330	0.16977	
	Overweight	0.58	0.15	0.3191	0.02811	0.12570	
	Obese	0.55	0.00	0.3120	0.02635	0.12360	
	Highly obese	1.54	0.16	0.4508	0.06655	0.30495	
NK cell counts	Underweight	0.98	0.09	0.3187	0.05013	0.24042	0.085 ^{NS}
	Healthy	0.43	0.13	0.2382	0.01825	0.09304	
	Overweight	0.88	0.10	0.2673	0.03701	0.16550	
	Obese	0.40	0.00	0.2307	0.02306	0.10816	
	Highly obese	0.69	0.15	0.3419	0.03433	0.15733	

HS, highly significant ($P < 0.01$).

S, significant ($P < 0.05$).

NS, not significant.

The mean T-cell counts in blood (Table 11) for both the moderate and high-risk groups are higher than those for the low-risk group. The ANOVA one-way test showed that these differences were statistically significant. Using the Tukey post hoc multiple comparisons test (Table 13), the moderate risk group had a significantly higher cell count in comparison with the low-risk group, whereas the high risk group was not significantly different.

The mean of CD4 cell counts in the blood (Table 11) were higher in both the moderate and high-risk groups when compared with the control group, and the ANOVA test showed that there was a significant relationship between CD4 cell counts and WHR. The Tukey post hoc test was used for the multiple comparisons between means. As shown in Table 13, the moderate risk group had significantly higher cell counts

than the low-risk group, while no significant difference resulted for the high-risk group in comparison with the control.

There were no significant relationships between WHR and B-, NK-lymphocyte cells counts, nor with CD8, CD19 B, and CD56 + CD16 NK cells in blood for both risk groups compared to the control group (Tables 11 and 12).

The ANOVA one-way test showed (Table 12) that the mean values of CD4 percentages and CD4/CD8 ratios were statistically different between the WHR groups. For multiple comparisons testing, the Tukey post hoc test was used since the data for CD4 are homogeneous. The CD4/CD8 ratios data are not homogeneous, but the Tukey test was also used here, because the tests for non-homogeneous data do not show differences between groups and thus

Table 7. Descriptive statistics and test of significance for lymphocyte subset percentages and BMI

	BMI	Max	Min	Mean	SE	SD	P-value
CD4 T cell percentage	Underweight	55	20	42.5833	1.61393	7.90661	0.480 ^{NS}
	Healthy	58	31	44.2692	1.09342	5.57536	
	Overweight	55	36	45.1304	1.11459	5.34538	
	Obese	64	32	44.04	1.45084	7.25419	
	Highly obese	59	37	46	1.30931	6.5	
CD8 T cell percentage	Underweight	43	16	29.5471	1.1841	5.80089	0.643 ^{NS}
	Healthy	39	16	28.0769	1.14478	5.83728	
	Overweight	41	15	27.6522	1.23616	5.92843	
	Obese	45	21	30.4	1.2	6	
	Highly obese	72	14	28.0476	2.56392	11.74937	
CD19 B cell percentage	Underweight	22	5	11.6667	0.8548	4.18763	0.347 ^{NS}
	Healthy	32	6	13.0769	1.14747	5.85097	
	Overweight	25	8	12.6957	0.84216	4.03884	
	Obese	19	6	11.88	0.68391	3.41955	
	Highly obese	65	7	15.3333	2.61072	11.96383	
CD65 + CD16 NK cell percentage	Underweight	39	3	11.4583	1.65282	8.09712	0.381 ^{NS}
	Healthy	18	6	10.5	0.66852	3.40881	
	Overweight	20	6	10.4783	0.75671	3.62904	
	Obese	21	2	8.92	0.79983	3.99917	
	Highly obese	24	5	11.619	1.09648	5.0247	
CD4/CD8 ratio	Underweight	3.06	0.71	1.5256	0.11036	0.54064	0.226 ^{NS}
	Healthy	2.90	0.84	1.6653	0.09648	0.49195	
	Overweight	2.84	0.90	1.7286	0.10727	0.51447	
	Obese	2.91	0.71	1.5424	0.11079	0.55397	
	Highly obese	3.71	0.58	1.8861	0.17557	0.80456	

NS, not significant.

Table 8. Multiple comparisons between mean lymphocyte and CD4 cells counts for the healthy group and other groups

	BMI (H)	BMI (Cat)		Mean differences (H-Cat)	SE	P-value
Lymphocyte cell	Healthy	Underweight	Tukey	-0.50334	0.21944	0.155
		Overweight		-0.21769	0.22801	0.875
		Obese		-0.56960	0.22492	0.091
		Highly obese		-0.73150	0.22492	0.013 ^S
		CD4 cells	Healthy	Underweight	Tukey	-0.18397
Overweight		-0.11732		0.11834	0.859	
Obese		-0.18640		0.11526	0.490	
Highly obese		-0.38267		0.11674	0.012 ^S	

The mean difference is significant against healthy weight subjects at the 0.05 level.

S, significant ($P < 0.05$).

H, healthy; Cat, category.

cannot explain the highly significant result of the ANOVA one-way test. The Tukey post hoc multiple comparisons test shows (Table 13) that the mean values of CD4 percentages and CD4/CD8 ratios are significantly and highly significantly higher, respectively, in the moderate group when compared with the control group.

Discussion

The role of the cells of the immune system in health maintenance and disease prevention is well-established. It was the aim of this study to determine the effects of obesity on the immune cells counts in Saudi female university students.

Table 9. Descriptive statistics and test of significance for WBC, platelet, basophil, eosinophil, monocytes, and neutrophil cells counts for the WHR categories

	WHR	Max	Min	Mean	SE	SD	P-value
Platelet counts	Low risk	509	151	308.0000	8.64120	74.83495	0.085 ^{NS}
	Moderate risk	474	292	350.3000	16.63934	52.61823	
	High risk	4800	183	336.1154	13.99267	71.34890	
WBC counts	Low risk	11.4	3.00	6.5357	0.19651	1.70178	0.000 ^{HS}
	Moderate risk	12.4	6.10	9.2320	0.56451	1.78513	
	High risk	12	5.25	8.0858	0.38771	1.97693	
Basophil cell counts	Low risk	0.40	0.00	0.0387	0.00733	0.06344	0.418 ^{NS}
	Moderate risk	0.10	0.00	0.0636	0.01521	0.05045	
	High risk	0.10	0.00	0.0385	0.00973	0.04961	
Eosinophil counts	Low risk	0.70	0.00	0.1400	0.01577	0.13656	0.587 ^{NS}
	Moderate risk	0.30	0.00	0.1800	0.02906	0.09189	
	High risk	1.10	0.00	0.1692	0.04252	0.21683	
Monocyte counts	Low risk	0.80	0.20	0.4933	0.01701	0.14735	0.729 ^{NS}
	Moderate risk	0.70	0.20	0.5100	0.05667	0.17920	
	High risk	0.80	0.30	0.5192	0.02661	0.13570	
Neutrophil counts	Low risk	7.90	1.10	3.2867	0.15381	1.33207	0.000 ^{HS}
	Moderate risk	7.00	3.20	5.4100	0.38829	1.22787	
	High risk	7.10	2.00	4.5000	0.32125	1.63805	

HS, highly significant ($P < 0.01$)

NS, not significant.

Table 10. Multiple comparisons between the low risk group and other groups for WBC and neutrophil counts

	WHR (L)	WHR (Cat)		Mean differences (L-Cat)	SE	P-value
WBC	Low risk	Moderate risk	Tukey	-2.69627	0.59793	0.000 ^{HS}
		High risk		-1.55004	0.40422	0.001 ^{HS}
Neutrophil counts	Low risk	Moderate risk	Tukey	-2.12333	0.47162	0.000 ^{HS}
		High risk		-1.21333	0.31883	0.001 ^{HS}

The mean difference is significant against low risk subjects at the 0.05 level.

HS, Highly significant ($P < 0.01$).

L, low risk; Cat, category.

The findings of this study show that the platelet counts in blood increase highly significantly ($P = 0.009$) as the BMI increases, whereas there was no correlation between the WHR and the platelet counts. The highly significant relationship between obesity measured by BMI and the counts of the peripheral blood platelets is in agreement with Anfossi and co-workers,¹¹ who found an increase of the platelet counts in obese women. Darvall and co-workers¹³ suggested that the increase in platelet counts results from the action of IL-6, which is produced by adipose tissue and is associated with the BMI. Therefore, the observed increase in cardiovascular disease and inflammation in the overweight, obese, and highly obese may in fact be due to the increased platelet counts shown in this study and proven by other researchers.^{11,13}

Total white blood cell counts are highly significantly related to both the BMI ($P = 0.00$) and WHR

($P = 0.00$) categories. The total white blood cell counts are highly significantly increased in both the obese (mean \pm SD, 8.2762 ± 1.98017) and highly obese (8.4176 ± 2.00459) groups compared with the healthy group (5.9462 ± 1.60505). The white blood cell counts are also higher in both the moderate (9.2320 ± 1.78513) and high (8.0858 ± 1.97693) WHR risk groups compared with the low-risk group (6.5357 ± 1.70178).

This total WBC increase goes hand in hand with the increase in the blood neutrophil cell counts, which are highly significantly related to obesity measured by the BMI ($P = 0.00$) and WHR ($P = 0.00$). For obesity assessed by BMI, the neutrophil counts in the obese (4.7476 ± 1.56065) and highly obese (4.5952 ± 1.55257) groups are above the counts in the healthy group (2.9500 ± 1.27695). For obesity measured by the WHR,

Table 11. Descriptive statistics and test of significance for lymphocytes, and lymphocyte subsets cell counts for the WHR categories

	WHR	Max	Min	Mean	SE	SD	P-value
Lymphocyte cell counts	Low risk	6.00	1.30	2.5613	0.08936	0.77388	0.052 ^{NS}
	Moderate risk	4.80	2.30	3.1000	0.26708	0.84459	
	High risk	5.60	1.70	2.8692	0.15579	0.79436	
T lymphocyte cell counts	Low risk	4.82	0.88	1.9644	0.07330	0.63480	0.034 ^S
	Moderate risk	4.13	1.88	2.4949	0.22275	0.70440	
	High risk	4.18	1.26	2.1637	0.12229	0.62357	
CD4 cell counts	Low risk	2.53	0.48	1.1317	0.04277	0.37042	0.012 ^S
	Moderate risk	2.35	1.09	1.5075	0.11684	0.36949	
	High risk	2.56	0.62	1.2494	0.08376	0.42708	
CD8 cell counts	Low risk	2.17	0.29	0.7285	0.03397	0.29417	0.177 ^{NS}
	Moderate risk	1.53	0.41	0.8491	0.14634	0.46278	
	High risk	1.50	0.46	0.8419	0.05001	0.25501	
B cell counts	Low risk	0.79	0.10	0.3193	0.01679	0.14537	0.147 ^{NS}
	Moderate risk	0.55	0.14	0.3481	0.03838	0.12136	
	High risk	1.54	0.15	0.4034	0.05665	0.28888	
NK cell counts	Low risk	0.98	0.09	0.2719	0.01953	0.16916	0.212 ^{NS}
	Moderate risk	0.41	0.05	0.2295	0.03747	0.11848	
	High risk	0.69	0.15	0.3244	0.02942	0.14999	

NS, not significant.

S, significant ($P < 0.05$).**Table 12.** Descriptive statistics and test of significance for lymphocyte subset cell percentages

	WHR	Max	Min	Mean	SE	SD	P-value
CD19 B cell percentage	Low risk	32	5	12.5976	0.51501	4.66365	0.433 ^{NS}
	Moderate risk	16	6	11.6364	0.89719	2.97566	
	High risk	65	7	14.2308	2.15857	11.00657	
CD65 + CD16 NK cell percentage	Low risk	39	3	10.5732	0.57579	5.21401	0.131 ^{NS}
	Moderate risk	14	2	7.9091	1.15565	3.83287	
	High risk	24	5	11.6154	0.98618	5.02853	
CD4 T cell percentage	Low risk	58	20	44.0122	0.69095	6.25684	0.012 ^S
	Moderate risk	64	32	49.7274	2.56228	8.49813	
	High risk	59	36	43.1538	1.05056	5.35681	
CD8 T cell percentage	Low risk	43	15	28.6707	0.6189	5.60435	0.215 ^{NS}
	Moderate risk	54	14	25.8182	2.74961	9.11941	
	High risk	72	17	30.3462	2.00763	10.23696	
CD4/CD8 ratio	Low risk	3.06	0.71	1.6194	0.05471	0.49541	0.004 ^{HS}
	Moderate risk	3.71	0.71	2.2156	0.28206	0.93550	
	High risk	3.47	0.58	1.5644	0.11415	0.58207	

HS, highly significant ($P < 0.01$)S, significant ($P < 0.05$)

NS, not significant.

the neutrophil cell counts are also higher in the moderate (5.4100 ± 1.22787) and high-risk (4.5000 ± 1.63805) groups compared with the low-risk group (3.2867 ± 1.33207). Thus, it may be concluded that neutrophil counts are directly affected by obesity by increasing their circulating counts. For the other differential white

blood cells (basophils, eosinophils, and monocytes), there are no significant relationships between either one and the BMI or WHR.

The previous results are in partial agreement with other researchers,¹⁶ who found an increase in the concentration of leukocytes, monocytes, and neutrophils in

Table 13. Multiple comparisons between the low risk group and other groups for T and T_h lymphocyte counts

	WHR (L)	WHR (Cat)		Mean differences (L-Cat)	SE	P-value
T lymphocyte counts	Low risk	Moderate risk	Tukey	-0.53047	0.21490	0.040 ^S
		High risk		-0.19928	0.14528	0.359 ^{NS}
CD4 cell counts	Low risk	Moderate risk	Tukey	-0.37573	0.12934	0.012 ^S
		High risk		-0.11764	0.08744	0.373 ^{NS}
CD4 percentage	Low risk	Moderate risk	Tukey	-5.71508	2.02435	0.015 ^S
		High risk		0.85835	1.41895	0.818 ^{NS}
CD4/CD8 ratio	Low risk	Moderate risk	Tukey	-0.59616	0.1816	0.004 ^{HS}
		High risk		0.055	0.12729	0.902 ^{NS}

The mean difference is significant against low risk subjects at the 0.05 level.

S, significant ($P < 0.05$).

L, low risk; Cat, category.

obese women, whereas in this work only leukocytes and neutrophils were found to increase in obese women in comparison with non-obese women. These results are also in agreement with another study¹⁷ that found a positive correlation between total white blood cell counts and neutrophil counts each with BMI. This increase in WBC and neutrophils may be evidence for considering obesity as an inflammatory disease, since it is known that WBCs play a major role in inflammatory responses,¹⁸ and that neutrophils are mobile phagocytic cells, which are generally the first cells to arrive at the site of inflammation.

It is of interest to note in the results that both total WBCs and neutrophil counts have a higher count in the moderate risk group compared to the high-risk group. These results may indicate that the increase in counts is associated with fat distribution, since in the moderate risk group fat is accumulated more in the central area of the body, whereas in the high-risk group fat is accumulated in the lower area (hips). This confirms the relationship between these cells counts and central adiposity.¹⁷ This also confirms the widely accepted fact that central fat in women is especially dangerous and leads to cardiovascular diseases and other ailments. Also, it is important to point out that the BMI gives a very different assessment of overall obesity from the WHR, which is concerned with distribution of fat in the body. This may explain the differences in the results obtained in this study for the immune system cells.

Total blood lymphocyte cell counts are significantly associated with the BMI ($P = 0.012$) but not associated with the WHR. The lymphocyte cell counts are significantly higher in the highly obese (3.0238 ± 0.81235) group compared with the healthy group (2.2923 ± 0.6046).

Total blood T-cell counts are significantly associated with the WHR ($P = 0.034$) but not associated with the BMI. The T-cell counts are significantly higher in the moderate risk group (2.4949 ± 0.70440) than the low-risk group (1.9644 ± 0.63480). CD4-cell counts are significantly related to the BMI ($P = 0.03$) and the WHR ($P = 0.012$). For CD4 cells using the BMI, the highly

obese group (1.3981 ± 0.43850) has a significantly higher count of CD4 cells compared to the healthy group (1.0154 ± 0.28761). As for the WHR categories, the moderate risk group (1.5075 ± 0.36949) has a significantly higher count compared to the low-risk group (1.1317 ± 0.37042). The CD8-, B-, and NK-cell counts do not correlate with either the BMI or WHR.

The CD4 T-cell percentages and the CD4/CD8 ratios are related to the WHR significantly ($P = 0.012$) and highly significantly ($P = 0.004$), respectively. For CD4 T-cell percentages using the WHR, the moderate risk group (49.7274 ± 8.49813) had a significantly higher percentage of CD4 T-cells compared to the low-risk group (44.0122 ± 6.25674). For the CD4/CD8 ratio, the moderate risk group (2.2156 ± 0.9355) had a highly significant higher ratio compared to the low-risk group (1.6194 ± 0.49541). Both CD4 T-cell percentages and CD4/CD8 ratios did not correlate with the BMI. The CD8 T, CD19 B, and CD65 + CD16 NK cells percentages did not correlate with either the BMI or WHR.

The previous results are partially in agreement with other studies²⁰ that found an increase in the lymphocyte subset counts with obesity, except for NK-cells and T-cytotoxic/suppressor cells. In this present study, a significant increase in the T and CD4 lymphocyte subset counts was obtained with increasing obesity, but not for NK-, B-, and CD8-cells. Obesity was also shown^{6,20} to decrease the proliferation of B- and T-cells. Another research study on females²² agrees somewhat with the results obtained in the current study in that it found that higher CD4 and total lymphocyte counts were associated with overweight and obesity, while higher CD8 counts were associated with morbid obesity and no significant correlation existed between CD4, CD8 cell percentages, and CD4/CD8 ratios with the BMI. Some researchers²⁴ reject the effect of obesity alone on the lymphocyte subsets.

In animal studies,⁶ obesity was found to affect cell-mediated immune response. The number of functional T-cells decreased in the obese animals which resulted in

impaired immunity. These animal studies confirm the destructive effects of obesity on the immune system.

Therefore, from the above results, it may be concluded that obesity affects immunity in general, since the increase in white blood cell counts correlates highly significantly with increased BMI and WHR. It is also evident that cellular acquired immunity is enhanced by obesity, since the total lymphocyte and CD4 cell counts increase highly significantly and significantly, respectively, as obesity measured by BMI increases. Also, cellular immunity is significantly enhanced by an increase in WHR for the total lymphocytes and CD4 cells. The CD4 cell percentages and CD4/CD8 ratios also significantly increase as WHR increases. On the other hand, B-cell counts and percentages did not change with the BMI or WHR, which leads to the conclusion that humoral immunity is not affected by increasing body weight. Finally, all innate immunity cells measured in this study (basophils, eosinophils, monocytes, and NK cells) did not show any correlation with the BMI or WHR. Neutrophils are the only innate immunity cell to show a highly significant increase with the increase of the BMI and WHR and, as mentioned earlier, these cells are important in inflammatory responses, which are evident in the obese. Therefore, it may be concluded that innate immunity is not appreciably affected by overweight and obesity. Also, the significant increases in the platelets and WBC counts in the obese BMI group confirm the roles of these molecules in inflammation and agree with the consideration that obesity is an inflammatory condition.

It is evident from the previous discussion that obesity that is not associated with diseases, such as diabetes, arthritis, and cardiovascular diseases, may still remarkably affect cells of the immune system as shown in the study sample. In addition, even the young age of the subjects does not prevent the adverse effects of obesity on immunity, hence the risk of having serious health complications is remarkably higher in obese subjects, of any age, than the healthy weight ones.

It is recommended that larger studies be done on both females and males and at different age groups to determine whether there are gender-dependent variations in the immune responses to overweight and obesity. Additionally, future studies should also measure the activity of immune system cells in addition to cell counts, thus leading to a better understanding of the effects of weight on the functioning of the immune system, since the increase in cell numbers does not mean a higher associated activity.

Acknowledgements

The authors thank Mr Ahmad M. Alkassar for his help with flow cytometry, Mr Abdulaziz Abdulbaqi for help in the hematology work, and Ms Alaweyah H. Basleab for her efforts.

Funding

This study was partially funded by King Abdulaziz City of Science and Technology (reference number: GSP-17-137).

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