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RESEARCH ARTICLE
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Insulin, Leptin and Ghrelin are Correlated with Sex but Not with Age in Healthy Subjects: A Cross-sectional Study

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ABSTRACT

Over the past ten years, there has been a significant global increase in the prevalence of obesity. Obesity can lead to several complications, such as metabolic problems, hormonal imbalances, cardiovascular diseases, and type 2 diabetes. The purpose of this study was to assess the connection between hormones, health biomarkers, waist circumference, and body weight. One hundred male and female participants in a cross-sectional study were included. Measurements of height, weight, and waist circumference were taken. A bioelectrical impedance analyzer was used to determine the percentage of total body fat. Measurements of % HbA1c, total cholesterol, triglycerides (TG), insulin, fasting blood glucose (FBG), ghrelin, and leptin were made using venous blood samples. Additionally, blood pressure was measured. Of the individuals, 27% were fat and 40% were overweight. About 57% of the participants had high body fat percentages, and 50% of them had high waist circumferences. Males had a greater waist circumference ($P<0.05$). In comparison to females, males showed significantly greater levels of TG, % HbA1c, fasting blood glucose, and lower levels of HDL. Leptin levels were considerably ($P<0.05$) higher and ghrelin levels were lower in females. In elderly participants, both diastolic and systolic blood pressure increased significantly ($P<0.05$). Of the participants in our study, 67% were overweight or obese. In summary, men were more likely to have diabetes, abdominal obesity, and dyslipidemia, which increases the risk of cardiovascular illnesses. Finally, females exhibited higher levels of hormones that regulate appetite than males did, and age was linked to higher blood pressure.

Keywords: Body weight, Diabetes, Glucose, Hormones, Lifestyle, Obesity

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INTRODUCTION

Obesity has a dramatically increased prevalence over the last 30 years of which 650 million people worldwide exceed the body mass index (BMI) of 30kg/m^2 (WHO, 2023). Globally, the prevalence of obesity nearly tripled between 1975 and 2016, and the percentage of obese adults worldwide was about 13% (11% of men and 15% of women (WHO, 2000a). Over 2 billion persons who were 18 years of age or older were overweight in 2016 (WHO, 2023). The high prevalence of a sedentary lifestyle is a major environmental factor contributing to the development and maintenance of obesity and overweight in Western societies (Rogerson et al., 2016). The prevalence of obesity among Jordanian adults is increasing greatly with age (Khader et al., 2008). A study conducted in Jordan

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in 2017 reported that the age-standardized prevalence of obesity was 60.4% among Jordanian males and 75.6% among Jordanian females, according to the International Diabetes Federation (IDF) criteria. While nearly three-quarters of men and women were overweight or obese (Ajlouni et al., 2020). Obesity prevalence among men and women over the age of 60 years has increased significantly during the last decade (Khader et al., 2008) and 41.5% of all obese adults were for those who are 60 years old or older (Stierman et al., 2017). In a study conducted among teenagers, it showed that general and abdominal obesity were prevalent in 41.3 and 46.9% of the population, respectively (Kerkadi et al., 2019).

In the United States, among male adults, obesity and overweight percentages reached 38 and 74.7% in 2015–2016 respectively (Youfa et al., 2020). On the other hand, since 1999, women's obesity/overweight prevalence has been steadily rising, reaching 41.5 and 68.9% respectively in 2015-2016 (Youfa et al., 2020). According to Yusuf et al. (2020), a study conducted on 30.6 million children in the United States revealed that 9.5 million (31%) of them were overweight or obese.

Waist circumference is a better estimator to determine visceral fat "abdominal obesity″ around accessory organs. Consequently, it is considered a more accurate predictor of cardiovascular risk, type 2 diabetes, and metabolic syndrome.

According to research, obesity is linked to a higher mortality rate as well as several diseases and conditions, such as diabetes, coronary heart disease, hypertension, dementia, Alzheimer's disease, stroke, and gastrointestinal disorders such as gallbladder disease, infertility, and cancer (Kinlen et al., 2018). The Global Burden of Disease study estimates that 4.7 million premature deaths in 2017 were related to obesity (Rennie et al., 2005). Obesity is caused by an intricate combination of genetic susceptibility and behavior. Numerous behavioral risk factors have been suggested, including high-density meals, excessively sweetened beverages with added sugar, portion sizes, eating habits, sedentary behavior, and low levels of physical exercise (Jebb, 2004). According to some studies, genetic and epigenetic changes that affect how the body's metabolic pathways function and how neurons and hunger centers are regulated are the root causes of obesity (Grill and Kaplan, 2002). Hundreds of these genetic variations, each having a small impact together, make some of us slightly more prone to gaining weight. Scientists have also discovered a mutation in a gene called the obese gene (ob), where the gene codes for the leptin hormone, secreted into the bloodstream by fat cells, and causes the feeling of satiety. It has been demonstrated that by activating receptors in brain stem and hypothalamic neurons, circulating leptin reduces food intake and increases energy expenditure (Zigman et al., 2003; Sitar-Tăut et al., 2021).

Several studies suggested that appetite hormones like leptin and ghrelin are the main hormones that work together but in opposition to one another, regulating each other and influencing feelings of hunger and appetite (Makris et al., 2017).

Ghrelin's exact function in the pathophysiology of obesity is still being researched, however, it is found that instead of ghrelin sensitivity, the issue with ghrelin is its excessive production regardless of food ingestion. There may be a variety of causes for this, from ghrelin gene dysfunction to the development of antibodies to peptide receptors that block ghrelin's activities, all of which result in obesity (European Health Examination Survey, 2013).

Recently, Jordanian adults tended to have many unhealthy eating practices such as overeating fast food which could significantly contribute to their body weight and increase the risk of many metabolic diseases and disorders. Thus, we have conducted this study to investigate if there is a relation between body weight, waist circumference, levels of leptin, triglycerides (TG), total cholesterol (TC), ghrelin, insulin, glycated hemoglobin, fasting blood glucose, and blood pressure. Therefore, the objectives of this study were to assess overweight and obesity in an institution's employee who relatively has a low level of physical activity due to their job nature and to evaluate the role of gender and age on anthropometric measurements and health biomarkers such as glucose, lipid profile, % HbA1c, insulin, leptin, and ghrelin.

MATERIALS & METHODS

Ethics

This study met the criteria set by the Institutional Review Board (IRB# 15/87/2018) for the protection of human research subjects committee in the King Abdullah University Hospital (KAUH) and at Jordan University of Science and Technology (JUST).

Study Design and Participants

This cross-sectional study was conducted on 105 JUST employees from different departments; their ages ranged between 24 and 57 years, and the mean was 40.5 years.

The Human Resources Department of JUST provided the researcher with a list of all employees and the anticipated participants were set to be chosen randomly by selecting subjects with odd numbers 1, 3, 5, 7, and 9 of every Department list regardless of age and gender. Subjects were visited at their working location, and they were informed about the study and its objectives. Subjects who agreed to participate in the study signed a written consent form and they were told that all information is confidential. Questionnaires were filled out on the same day. The questionnaire was validated, and it included questions about dietary habits, physical activity, sleeping patterns, drug use, health conditions, and lifestyle practices. Each question was read and explained verbally to guarantee understanding and accurate answers.

Inclusion and Exclusion Criteria

Female and male employees who worked for JUST at any Department were included in the study unless they were on anti-inflammatory drugs, statins, and antibiotics or had previously had surgeries. Females were also excluded if they were pregnant, lactating, or using oral contraceptive drugs. The overall study subjects after exclusion were 100 subjects.

Anthropometric Measurements and Body Composition

Weight and Height

Weight and height were measured using anthropometric measures in accordance with World Health Organization guidelines (2000). Using a digital scale (Tanita SC330ST), body weight was measured while subjects were dressed comfortably and without shoes. We used a measuring rod (Seca, Germany) to measure height. The subjects were instructed to position their heads in the horizontal Frankfort plane. According to Asmar et al. (2010), all participants were instructed to remove their shoes while standing with their heels together and their toes apart, ensuring that as much of their backs, shoulders, buttocks, and heels touched the backboard.

Body Mass Index

The formula for calculating Body Mass Index (BMI) is kg/m^2, which is the ratio of weight (in kilograms) to height (in meters squared). The WHO's body mass index classifications were applied in this investigation.

Waist Circumference

A non-stretchable circumference measuring tape (SECA 203, Germany) was used to measure waist circumference (WC) to the closest centimeter. The WHO states that the tape deployment site was halfway between the lower rib border and the iliac crest. Waist circumference was considered high if it is more than 102 cm, and 88 cm in males and females respectively based on WHO criteria.

Body Composition

The body fat content was assessed using a Body Composition Analyzer (TANITA, BC 418). Body fat percentage cut-off points, depending on gender and age, are classified into three levels (healthy body fat percentage, high body fat percentage, and very high body fat percentage (obese)), as shown in Table 1.

Table 1: Body fat percentage cut-off points for males and females depending on age

Body Fat (%)	Age (year)					
	Less than 39		40-59		60 and More	
	Males	Females	Males	Females	Males	Females
Healthy body fat	8-20	21-33	10.1–22	23.1-34	12.1-25	24-36
High body fat	20.1-25	33.1-39.5	22.1-28	34.1-40	25.1–30	36.1-42
Very high body fat (Obese)	≥ 25.1	≥39.6	≥28.1	≥40.1	≥30.1	≥42.1

Cut-off points based on NIH/WHO BMI guidelines as reported by (Gallagher et al., 2000). Biochemical Analysis.

Biochemical Tests

Venous 10mL blood samples were drawn from the study subjects after fasting for at least 8 hours by a practitioner nurse from the health center of JUST. Each collected blood sample was divided into two tubes; EDTA and Z-Clot activator tube. EDTA tubes were used for the quantitative determination of glycosylated hemoglobin (Beckman Coulter AU analyzers).

Meanwhile, the Z-Clot activator tubes were left for at least 30min to allow clotting and then centrifuged at 4000rpm. An Aliquot of serum was stored at -40°C in vacutainer tubes before the biochemical assay. The Immuno-turbidimetric test was used to analyze ghrelin and leptin hormones (Beckman Coulter AU analyzer). In addition, serum was used to analyze other biomarkers such as triglycerides, cholesterol, fasting blood glucose, and insulin levels.

Blood Pressure

Blood pressure (BP) was measured by the same nurse using the automated oscillometric device, Omron M3 Iinrellisense (HEM-7051) which is a valid device according to the international protocol (Neuhauser et al., 2013). The first reading was measured after the submission of the questionnaire and while subjects were at rest according to a standardized protocol (Musaiger et al., 2013). The individuals' left arm was used to measure blood pressure while they were seated calmly for five minutes to reduce anxiety. They also sat with their feet flat on the ground, their backs supported, their right arms supported, and their left cubital fossa at heart level. Every subject provided three readings on three separate days. Three readings were taken, and the average was noted. The beginning of the "tapping" KSs (K1) and the fifth KS (K5) indicated the diastolic blood pressure, whereas the KSs' absence indicated the systolic blood pressure. The BP percentiles were calculated based on the recorded mean values of blood pressure.

Questionnaire

The study questionnaire has been adopted from Musaiger and his colleagues' study (Musaiger et al., 2011), which has been used to describe the dietary and lifestyle habits in Bahrain after we have a slight modification to test our intended variables. The questionnaire was composed of personal history, socio-demographic data, and parents' educational level as well as lifestyle data which include dietary habits and physical activity (Bredella, 2017).

Statistical Analysis

Data processing and analysis were performed using SPSS, version 19 by Chicago, Inc., a statistical package for social sciences. For categorical data, the frequency distribution was used to characterize the characteristics of the respondents, and for continuous variables, the mean and standard deviation were utilized. Ghrelin, the dependent variable, was not normally distributed and did not become so even after logarithmic treatment. For this dependent variable, nonparametric statistics (the Mann-Whitney U-test or the Kruskal-Wallis test) were employed. To investigate the effects of the relevant factors, multivariate analysis of variance (MANOVA) was employed. To find the difference between the variables, a post-hoc Least Significant Difference (LSD) MANOVA was used. P-values less than 0.05 were regarded as statistically significant.

RESULTS

About 54% of the study samples were males. Most of the subjects were educated (92%) since they work in different Departments of an academic institution. About 52% of subjects were living in cities. Only 39% of subjects reported a family income of more than 1120 $US. Only 46% of the subjects were smokers. About 68% of subjects have followed a diet at least once in their lives.

Table 2 shows the anthropometric characteristics of the subjects in comparison to gender. Thirty-three percent of subjects had a normal BMI of 18.5-24.9 kg/m², while 40% of subjects were considered overweight and about 27% of all subjects were obese. Regarding waist circumferences, 49% of subjects had a normal waist circumference and 51% were described to have a high waist circumference. Males and females did not differ significantly in BMI; however, males had significantly lower body fat percentage and a significantly larger waist circumference than females (P<0.05).

Table 2: Anthropometric characteristics of the study Subjects (n=100).

Measurement	Total n (%)	Males n (%)	Females n (%)	P value
	Body Mass Index (BMI) (Kg/m²)‡			
Normal	33(33.0)	18(37)	15(28)	0.09
Overweight	40(40.0)	20(37)	20(33)	
Obese	27(27.0)	16(29)	11(39)	
	Waist circumference (WC)(cm)§			
Normal	49(49.0)	22(40)	27(59)	0.04
High	51(51.0)	32(60)	19(41)	
	Body Fat (%)			
Low fat percentage	5(5.0)	3(5)	2(4)	0.03
Healthy fat percentageΩ	38(38.0)	27(50)	11(24)	
High fat percentage	57(57.0)	24(45)	33(72)	

‡ Normal weight (18.6-24.9kg/m²), overweight (25- 29.9kg /m²), obese (≥ 30kg /m²). § High waist circumference when >102cm in males, and >88cm in females (WHO, 2000b); Ω Healthy: Age 30-39 (7.1-20% for males, 21-33% for females), Age 40-59 (10.1-22% for males, 23.1-34% for females), Age 60 and above (12.1-25 for males, 24-36 for females). Over-Average: Age 30-39 (20.1-25% for males, 33.1-39.5% for females), Age 40-59 (22.1-28% for males, 34.1-40% for females), Age 60 and above (25.1-30% for males, 36.1-42% for females). Obese: Age 30-39 (≥25.1% for males, ≥ 39.6% for females), Age 40-59 (≥28.1% for males, ≥40.1% for females), Age 60 and above (≥30.1 for males, ≥42.1% for females).

Our study results indicated significantly higher levels of TG and lower levels of HDL in males than in females. A significant difference (P=0.04) in HbA1c levels was revealed also between males and females. Males had significantly higher (P<0.05) HbA1c and FBG values than females. A clear trend is demonstrated in Table 3 with higher diastolic pressure among male participants compared to females, but it does not reach a significance level. The mean serum levels of leptin and ghrelin in males and females respectively were significantly different (P<0.05) (Table 3).

Table 4 shows the effect of age on the mean values of blood metabolites, hormones, and blood pressure. The statistical analysis shows a significant difference (P<0.05) only in blood pressure between age groups in both systolic and diastolic readings. None of the other blood metabolites were significantly different according to age.

DISCUSSION

In Jordan, like all the other countries, obesity is considered a growing epidemic with insufficient pieces of evidence about preventive strategies. Our study sample revealed that 66% of males and 72% of females were overweight and obese. This is an alerting rate that is worth more studies and preventive strategies. Khader et al. (2008) reported that 59.8% of the females and 32.7 of the males are overweight or obese in Jordan. Their reported prevalence was 20 years ago meaning; that with years the prevalence is increasing dramatically. Our research indicates that the bodily types of men and women differ. Men possess more lean mass, while women have more fat mass. Men are more likely to have fat throughout their trunk and abdomen, while women usually gain adipose tissue across their hips and thighs (Kodoth et al., 2022). Recently, it has been determined that menopausal women experience changes in their body composition, including a notable acceleration of lean mass loss and a rise in body mass, as well as a transfer of fat to the abdomen observed due to a decline in estrogen levels. Sixty to seventy percent of women in their mid-life experience weight gain as a sign of menopause. Women gain 1.5 pounds annually between the ages of 50 and 60 throughout the midlife period (Stevens et al., 2010). Similar gender-based variations in waist circumference were noted in non-Caucasian ethnicities. Men's waists in the working-class Chinese population of Hong Kong were found to be 5.9cm bigger than women's (Pedro, 2019).

Table 3: Mean of blood metabolites, hormones and blood pressure in males and females.

Blood tests	Sex		P value
	Male (n=74)	Female (n=26)	
	Lipid profiles		
Total Cholesterol (mmol/L)	5.55±0.11	5.57±0.19	0.75
Triglyceride (mmol/L)	2.39±0.24 [a]	1.33±0.17 [b]	0.03
HDL (mmol/L)	0.95±0.02 [a]	1.22±0.05 [b]	0.01
LDL (mmol/L)	3.79±0.09	3.93±0.17	0.92
	Glucose indices		
Insulin (uu/mL)	11.14±1.06	9.11±1.65	0.26
% HbA1c	5.54±0.11[a]	5.154±0.06[b]	0.04
Fasting Blood Glucose (mg/dL)	120.19±4.89[a]	104.84±2.16[b]	0.05
	Blood Pressure		
Systolic (mmHg)	81.44±1.99	72.14±3.13	0.29
Diastolic (mmHg)	130.0±2.40	120.55±4.40	0.08
	Hormones		
Leptin (ng/mL)	3.92±0.17[a]	4.60±0.27[b]	0.04
Ghrelin (ng/mL)	11.70 (8.77-12.20)[a]	10.60 (7.58-11.10)[b]	0.03

All values are mean ± SEM. Values were determined with MANOVA (P<0.05) after adjustment for age, gender smoking, and exercise level. Ghrelin median minimum/maximum.

Table 4: Mean of blood metabolites, hormones and blood pressure in different age groups.

Blood test	Age (year)		P value
	25-40 (n= 54)	>40 (n= 46)	
	Lipid profiles		
Total Cholesterol (mmol/L)	5.53±0.12	5.59±0.15	0.95
Triglyceride (mmol/L)	1.83±0.22	2.38±0.32	0.59
HDL (mmol/L)	1.05±0.03	1.00±0.04	0.57
LDL (mmol/L)	3.95±0.09	3.96±0.13	0.72
	Glucose indices		
Insulin (uu/mL)	8.46±0.86	12.52±1.58	0.29
% HbA1c	5.37±0.14	5.48±0.09	0.85
Blood Glucose (mg/dL)	115.33±5.30	117.26±5.25	0.84
	Blood Pressure		
Systolic (mmHg)	74.02±2.10[a]	85.39±2.60[b]	0.05
Diastolic (mmHg)	121.86±2.20[a]	133.44±3.73[b]	0.02
	Hormones		
Leptin (ng/mL)	4.05±0.24	4.13±0.20	0.71
Ghrelin (ng/mL)	10.70(7.58-11.10)	10.70(8.01-11.20)	0.60

All values are Mean±SEM. Values were determined with MANOVA (P<0.05) after adjustment for age, gender smoking, and exercise level. Ghrelin median minimum/maximum.

Obesity is a risk factor for many diseases. Diabetes mellitus, hypertension, and many cardiovascular diseases are just a few examples. Serum HDL, total cholesterol, and triglycerides are considered good indicators for the health of the heart and arteries. The significant difference between males and females in HDL and TG levels represented in our study agreed with a study done in Portugal among 161 individuals randomized from the community (77 men and 84 women). The study showed that the overall prevalence of dyslipidemia was significantly higher in males than in females for total cholesterol, triglycerides, and low-density lipoprotein cholesterol but, was lower for high-density lipoprotein cholesterol in females than in males (Barton, 2013).

Recent clinical investigations have revealed the potential advantages of natural estrogen, including improved blood circulation, suppression of inflammation, prevention of plaque progression, and affecting lipid metabolism; increased HDL, lowered LDL cholesterol levels, and lowered triglyceride levels (Huang et al., 2021). Our results demonstrated lower levels of HbA1c in females when compared to males. Male HbA1c levels were considerably higher than female HbA1c levels for the two age groups of 30-39 and 40-49 years, according to another study (Qinglin et al., 2016). Similarly, in a study conducted on Chinese adults with no prior onset of DM, a significant difference in hemoglobin A1c values was revealed (Anish et al., 2013).

In correspondence with Anish et al. (2013) after overnight fasting (10 hrs at least), fasting blood glucose levels were significantly lower among women (Tramunt et al., 2020). More studies have been done recently to comprehend how gender differences affect the pathophysiology of many diseases, including metabolic disorders like diabetes (Nauli and Matin, 2019). Males are more likely than females to acquire obesity, insulin resistance, and hyperglycemia, whereas females have higher levels of insulin sensitivity (Nauli and Matin, 2019). Compared to pre-menopausal women, men are more likely to develop abdominal visceral fat (Janochova et al., 2019). Visceral adipose tissue makes up 20% of the total fat in the body and is located around the internal organs. Elmarakby and Sullivan (2021) have established a correlation between it and insulin resistance, hypertension, elevated triacylglycerol levels, a heightened risk of diabetes, and cardiovascular ailments.

The primary modifiable risk factor for cardiovascular disease morbidity and death globally is uncontrolled hypertension, and it can lead to stroke, heart attack, heart failure, and aneurysm (Alhawari et al., 2018). Several recent research comparing the mean difference in both systolic and diastolic blood pressure with BMI showed that blood pressure is greater in men than in women at similar ages (Olivera and Graham, 2023). Similar to humans, differences between males and females have been seen in animal models of hypertension. In 1949, it was discovered that male normotensive dogs had higher average blood pressure levels (up to 9mmHg) than female dogs, which was the first indication of sex variations in the control of blood pressure in animal models (Ostchega et al., 2020).

In recent studies, blood pressure was found to be similar in both sexes up until adolescence, but thereafter it dramatically increased in males compared to females of the same age (Cheng et al., 2022). When grouped by age, males continue to have higher rates of this condition. In the age group 18–39 years, the prevalence in males is 31.2%, whereas in females, it is 13.0%. In the age group 40–59 years, it is 59.4% in male's vs 49.9% in females, while over 60 years it is 75.2% in males and 73.9% in females (Cheng et al., 2022). Our findings confirmed the results of the previous author. Both systolic and diastolic pressure increased significantly in the older age group (>40 years) compared to the younger group (25-40 years). In accordance with our findings, a study conducted in China compared fasting serum leptin levels among 469 men and 773 women where the mean ± standard deviation serum leptin level was much higher in women than in men (Schorr et al., 2018). It is scientifically proven that the secretory organs of leptin are the adipocytes. Consequently, the difference in the body composition between males and females is related to the greater muscle mass in males than the muscle mass in females and the lower percentage of body fat among males.

Conclusion

Recently, obesity has been diagnosed as a major health problem worldwide as well as in Jordan. About 67% of our study subjects were overweight and obese. The goal of research may be to clarify the causes of this issue. To avoid obesity and lower the risk of numerous linked illnesses like diabetes, hypertension, and hyperlipidemia, it is important to better understand dietary practices, physical activity, and other lifestyle risk factors that may contribute to obesity. Males were more likely than females to have diabetes, dyslipidemia, abdominal obesity, and cardiovascular disease. Females exhibited higher levels of the hormones ghrelin and leptin. Age was linked to increased blood pressure. Finally, there is an urgent need to raise the awareness of decision-makers on the consequences of obesity to propose preventive strategies and programs that will help in controlling and avoiding obesity and being overweight.

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Conflicts of Interest Statement

The authors declare no conflict of interest.

Author Contributions

HS and BO: writing the original draft, editing, investigations, data analysis, and supervision. **MM:** writing the original draft and data collection; **BSO, NAl-Ba, LO, NAEl, and LAl:** writing and editing the final draft. All authors revised and approved the final version of the manuscript for publication.

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