Impact of Leptin Receptor Gene SER343SER Polymorphism on Obesity in Jeddah City

Sabah A. Linjawi and Rewaa A. Al-Sayed

Department of Biology, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia
slinjawi@kau.edu.sa

Abstract: Obesity is being described as a global epidemic because its prevalence globally in both the developed and the developing countries.Obesity is an excessive accumulation of body fat result from an interaction of genetic and environment factors. And from genetic factors leptin receptor gene which plays an important role in the regulation of body weight, regulation of energy and fat storage. Genetic variations of leptin receptor gene may play a role in the pathophysiology of human obesity. In this study, the association between the OB-R gene polymorphism and obesity in Jeddah city population was evaluated by determine the distribution of alleles frequency of the leptin receptor SER343SER polymorphism in 150 volunteers (71 males & 79 females) from Jeddah population. Each gender was divided into three groups according to BMI (underweight, normal "control" and obese). Genotypes were determined for all subjects by using polymerase chain reaction (PCR) and Cut followed by restriction enzyme.When comparing between genotype and body mass index (BMI), results demonstrated that there is a higher significant difference (P=0.000). This significant was observed when the genotype CC was compared with either TT or TC genotypes. In contrast, there was a significant increase in CC genotype (OR= 12.8, 95%CI: 1.15 -142.58, P= 0.03) compared to TT genotype in males which suggests that genetic polymorphisms (SER343SER) of leptin receptor gene may play a role in underweight males.
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1. Introduction

Obesity has reached epidemic proportions to its prevalence in many developed and developing countries. At its simplest level obesity can be defined as an imbalance between the energy that is ingested (Energy In) and the energy that is expended (Energy Out). Obesity and weight gain can be considered a major health problem increased in Saudi population over the past thirty years where the total obesity arrived in national study on Saudis adults in 2005 to 35.5%. **(Al-Nozha *et al.*, 2005; Al-Sultan *et al.*, 2006; Marti *et al.*, 2009; Bahathiq, 2010)**. According to World Health Organization (WHO) statistics, in Saudi Arabia to 2008 is estimated to 39.1% in females and 28.6% in males were obese. This value is higher than that reported in the United Kingdom, Australian, Americans and Italian populations this rising an alarming, especially among females **(Al-Nozha *et al.*, 2005; Bahathiq, 2010)**. So health authorities must take the necessary measures to fight obesity responsible for a lot of health problems and do health education campaigns of Saudi society about the health risks of obesity on the individual and the treatment of obesity by increasing medical and scientific researches to identify the causes of obesity, how to prevent it, improve eating habits and levels of physical activity to the community. During the last decade several studies have shown a close relationship between a hormone leptin and development of obesity.

The name leptin was derived from the Greek word leptos, meaning thin, because it induces weight loss when injected in mice (**Campfield *et al.*, 1995; Mouzon *et al.*, 2006).** This name was proposed by **(Halaas *et al.*, 1995**) for the fat-regulating hormone Leptin is an adipose-derived cytokine present in the circulation in amounts proportional to fat mass (**Mergen *et al.*, 2007**) encoded by the obese (Ob) gene localized on human and mouse 7 and 6 chromosomes, respectively. The Ob gene was first discovered in adipose tissue of the obese mouse through positional cloning. The full coding sequence contains 167 amino acids and represents a 21-amino-acid signal peptide and 146-amino-acid circulating bioactive hormone. In both human and mouse, the leptin gene is composed of three exons and two introns (**Bagchi and Preuss, 2007).**

The Ob gene is highly conserved among vertebrates. Mouse leptin shares 84% sequence identity to the product of the human Ob gene. When mutated Ob gene, the Ob gene is no longer deliver its appetite-suppressing message. The mice consequently develop a syndrome that resembles extreme obesity and type 2 diabetes in humans **(Munsch and Beglinger, 2005).**

The level of circulating leptin is directly proportional to the total amount of fat in the body and body mass index (BMI), the greater levels of leptin found in individuals with more fat and a decrease in leptin levels with weight loss **(Maffie *et al.*, 1995)**.

Serum leptin levels are affected by nutritional status, fasting reduces leptin levels by approximately 30% while excessive food consumption leads to an increase in the secretion of leptin by 50%. Leptin works on reduce appetite and increase energy expenditure by binding to its receptor, encoded by the diabetes (db) gene. The leptin receptor was first isolated from the mouse choroid plexus using expression cloning **(Tartaglia *et al*., 1995)**.

In humans there are six isoforms can be divided into three classes: secreted (Ob-Re), short (Ob-Ra, Ob-Rc, Ob-Rd and Ob-Rf) and long forms (Ob-Rb). The isoforms are all generated by alternative splicing from a single gene (leptin receptor gene) located on chromosome 1p **(Gotoda *et al*., 1997; Thompson *et al*., 1997)**. The genomic structure of the human leptin gene spans over 70 kb and includes 20 exons **(Thompson *et al*., 1997)**. Leptin is predominantly produced by adipocytes, but leptin receptors are located in many tissues, in organs involved in energy storage, metabolism and digestion, such as skeletal muscle, adipose tissue, pancreas, stomach, small intestine, colon and liver. There are many peripheral tissues as sites of Ob-R, such as brain (the choroid plexus and hypothalamus), the lungs, kidneys, adrenals, ovaries, uterus and testes. Ob-R can be also found in tissue related to immunity, such as spleen, thymus, lymph nodes haematopietic cells and T-cells **(Frühbeck, 2001; Margetic *et al*., 2002; Bjorbaek and Kahn, 2004; Bagchi and Preuss, 2007)**.

Single nucleotide polymorphisms (SNPs) are a single base substitution of one nucleotide with another throughout the human genome, in both coding and non-coding regions. This type of polymorphisms is the most common variations among individuals **(Venter *et al*., 2001)**. Several (SNPs) have been described in the human leptin receptor gene **(Considine *et al*., 1996; Matsuoka *et al*., 1997)**. The five of the more common polymorphisms are: (Lys109Arg, Gln223Arg, Ser343Ser, Lys656Asn, Pro1019Pro) **(Gotoda *et a*l., 1997)**. They were distributed across the coding region, four being in the extracellular and one in the intracellular portion of the human Ob-R protein. Studies have focused on the leptin receptor polymorphisms that are considered one of the genetic factors that cause obesity, in an attempt to determine its role in the development of obesity **(Al-Sultan, *et al*., 2006)**. One of the polymorphism of leptin receptor is SER343SER that resulted by silent mutation gene across the replacement of base nitrogen of thymine to cytosine (T-C) AGT/AGC at the site 1029 on exon 9. This polymorphism at codon 343 located in the extracellular region of leptin receptor is within the region encoding. This replacement gave the amino acid serine again because it has six genetic codes (TCT- TCC- TCA- TCG- AGT - AGC).The SNP SER343SER (T> C) has been studied, in the number of population, several studies no have associated for this variant with obesity.

This study aims to detect the presence of SER343SER leptin receptor polymorphism in obese patients to both genders in Jeddah city. To investigate the frequency of alleles of SER343SER in Jeddah population, extract DNA from blood samples, and digest the PCR product with the restriction enzyme *MluI* to detect alleles of SER343SER and statistically analyzed by using SPSS software.

2- Materials and Methods

Human subjects:

This study was approved by the ethical Committee (unit of biomedical ethics) from King Abdulaziz University; all participants gave their written informed consent of their participation. The study included 150 adult participant (71 males and 79 females) randomly selected from Jeddah city population, aged 15 to 70 years .The blood samples of the subjects were collected from King Abdulaziz University Hospital in Jeddah by Noor Hussin. All the participants underwent complete physical examination. At the time of blood collection, information was recorded for all subjects, including height, weight, waist (WC), and hip circumferences (HC) only for females. All volunteers were asked to answer a questioner about family history of obesity and genetic diseases. Exclusion of pregnant women and people who use any treatment course in this study. Each gender was divided into three groups: underweight, normal and obese according to BMI. Classification of BMI for adults according to the World Health Organization (WHO) (Healthy BMI < 25 and Obese BMI ≥25). The practical works of this research were conducted at the Biology Postgraduate Studies laboratories, sixth building at King Abdulaziz University, Jeddah.

Methodology:

Genetic Analysis:

Genomic DNA was extracted from whole blood which stored in EDTA coated tubes by using Qiagen-QIAamp DNA Blood Mini Kit. The concentration of genomic DNA was determined by the quantitative method, which is based on the optical density measurement. Was quantified using spectrophotometric analysis using 6800 UV/Vis Spectrophotometer (JENWAY, UK). The purity was determined by calculation the ratio of absorbance at 260 nm to absorbance at 280 nm (A260/A280). Pure DNA should have an A260/A280 ratio of 1.7 - 1.9.

Amplification for SER343SER gene

Genotyping of the Ob-R polymorphism was carried out using the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP)assay with previously described primer pairs **(Gotoda**

***et al.*, 1997)**. Forward primer: 5'- ACT TTC CAC CTA AAA TTC TGA CAC G -3' and Reverse primer: 5'- TTC CTT CTT ATA GAT GCA GTG 3'. PCR amplification products were obtained using (Maxima Hot Star Green Master Mix Kit) a final volume of 50µl and prepared as [3µl genomic DNA (2µg/µl), 25µl Hot Star Green Master Mix, 20µl nuclease free water, 1µl of each primer] in a Thermal cycler (mastercycle personal, Eppendorf,Germany). The amplification conditions were as follows: initial denaturation at 95°C for 5 min followed by 35 cycle of denaturation at 94°C for 30s, annealing at 50°C for 45s and extension at 72°C for1 min, followed by final extension at 72°C for 5 min **(Gotoda *et al.*, 1997)**. The PCR product was purified by using the isolate PCR kit (Bioline Inc., USA). The DNA bands were Visualized under UV light and photographed using gel documentation (G: BOX EF, Chemi, ChemiHR16, ChemiXT16 and ChemiXL Camera Hardware, Syngene, USA). The ethidium bromide (fluorescent dye) intercalates between bases of DNA causing the visualization of the bands. The gel was then photographed.

Genotyping of rs1805134SNP in SER343SER gene:

The resulting DNA fragment was 68 bp in length **(Gotoda *et al.*, 1997)**. The genotypes for this SNP were determined by restriction fragment length polymorphism (RFLP) procedure. They were prepared as follows: in a labeled clean and dry Eppendorf tubes 17µl of PCR product, 2µl of 10X NE Buffer 3, mixed by pipetting. Finally, 1µl of restriction enzyme (*MluI*) were added. The tubes were incubated for overnight at 37^0C followed by heat inactivation for 20 minutes at 65˚C. After that, the genotypes were resolved after running it on 3% (w/v) agarose gels electrophoresis.

Statistical Analysis:

All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 16.0 (SPSS Inc., Chicago, IL, USA). Descriptive data were given as mean ± standard deviation (SD).The associations between BMI and other parameters with genotypes were performed using analysis of independent samples T-test .A case-control study was designed to compare patients (who had underweight or obese) with control (who had normal weight). The Fisher's exact test and the Chi-square test were applied to test the association between genotypes and clinical groups. Contingency analysis was applied to calculate the odds ratio (OR) at 95% confidence interval (CI) was used to estimate the relative risk and strength of association for their various genotypes or their combinations. Statistical significance was defined as the probability of $P \leq 0.05$ (two-sided). Hardy-Weinberg Equilibrium was tested by goodness-of fit $\chi 2$ test to compare the observed genotypes frequencies to the expected genotypes frequencies for all data, chi-square test featuring one degree of freedom.

3- Results

Table 1 shows all data; there were a significant difference between the normal group, with both underweight group and obese group in weight and BMI ($P< 0.05$).Table 2 shows males' group data; there were a significant difference between the normal group with both underweight group and obese group in weight and BMI ($P< 0.05$).Table 3 shows females' group data, which represents a significant difference between the normal group, with both underweight group and obese group in weight and BMI ($P< 0.05$). Also found a significant difference in age between the obese group and normal group ($P< 0.05$).

Figure 1 shows the genotyping results of SER343SER leptin receptor polymorphism. A single band of 68 bp shows the presence of allele T, while the presence of 2 bands of 43 and 25 bp shows the presence of allele C. There are 2 bands for the C allele because this product contains a digestion site for the *MluI* enzyme, which is absent when T is presence. Therefore, the PCR product containing the C allele is cleaved by *MluI* and produces 2 bands of low molecular weight.

Table 4 shows the genotypes distribution in all data for SER343SER polymorphisms. The genotype frequencies were 58.7% (n=88) TT homozygous, 34.7% (n=52) TC heterozygous and 6.7% (n=10) CC homozygous, with 76% and 24% T and C allele frequencies, respectively. The data with TT and CC genotypes were highly significant differences in weight (P=0.000) and BMI (P= 0.000), also with TC and CC genotypes were highly significant differences in weight (P=0.001) and BMI (P= 0.000). Data with TT genotype showed the higher values in weight (65.98±22.33) and BMI (25.25±7.79), while data with CC genotype showed the lowest values in weight (46.40±11.11) and BMI (17.83±0.81). The balance of homozygotes and heterozygotes observed was as predicated by the Hardy-Weinberg equation for these allele frequencies (Goodness of fit X2= 0.37, df = 1, P= 0.54).

Table 5 shows males' group data of the genotypes distribution for the SER343SER polymorphisms. The genotype frequencies were 56.3% **(*n*=40)** TT homozygous, 36.6 % **(*n*=26)** TC heterozygous and 7% **(*n*=5)** CC homozygous, with 75 % and 25% T and C allele frequencies, respectively. Males with TT and CC genotypes were significant differences in weight (P=0.02) and BMI (P= 0.001), also with TC and CC genotypes were significant differences in weight (P=0.04) and BMI (P= 0.009). Males with CC genotype showed the lowest values in weight (47.40±15.54) and BMI (17.75±3.17). The balance of homozygotes and heterozygotes observed was as

predicated by the Hardy-Weinberg equation for these allele frequencies (Goodness of fit X2= 0.07, df = 1, *P*= 0.78).

Table 6 shows females group data of the genotypes distribution for the SER343SER polymorphisms. The genotype frequencies were 60.8% **(*n*=48)** TT homozygous, 32.9% **(*n*=26)** TC heterozygous and 6.3% **(*n*=5)** CC homozygous, with 77% and 23% T and C allele frequencies, respectively. Females with TT and CC genotypes were significant differences in weight (*P*=.002) and BMI (*P*=0.00), also with TC and CC genotypes were significant differences in weight (*P*=0.003) and BMI (*P*= 0.002). Females with CC genotype showed the lowest values in weight (45.40±5.81) and BMI (17.90±2.16). The balance of homozygotes and heterozygotes observed was as predicated by the Hardy-Weinberg equation for these allele frequencies (Goodness of fit X2= 0.33, df = 1, *P*= 0.56).

Table 1 Descriptive of data for all volunteers (*n* = 150).

Variables	BMI class			*P* value[a]	*P* value[b]
	Underweight	Normal	Obese		
N	38(25%)	55(37%)	57(38%)		
Age (Years)	19.08±3.44	19.05±2.84	24.70±12.71	0.970	0.002*
Weight (Kg)	42.32±5.77	55.07±7.37	86.77±17.47	0.000*	0.000*
Height(m)	1.60±.085	1.62±.081	1.63±.101	0.288	0.525
BMI (Kg/m^2)	16.53±1.45	21.00±1.81	32.59±5.26	0.000*	0.000*

** highly Significance (*P*<0.05).Values are expressed as mean ± standard deviation (SD), and were compared by t-t est.BMI; Body mass index. *n*; no. of sample. *P*-value a: Underweight *vs.* Normal. *P*-value b: Normal *vs*. Obese.

Table 2: Descriptive of males group data (*n* = 71).

Variables	BMI class			*P* value[a]	*P* value[b]
	Underweight	Normal	Obese		
N	16 (22.5%)	27 (38%)	28(39.4%)		
Age (Years)	20.50±3.29	19.32±3.27	29.62±16.21	0.21	0.002**
Weight (Kg)	41.10±4.77	52.09±5.40	78.40±10.85	0.000*	0.000*
Height(m)	1.58±.06	1.57±.049	1.58±.05	0.65	0.71
BMI (Kg/m^2)	16.48±1.45	21.06±1.73	31.42±3.86	0.000*	0.000*

** highly Significance (*P*<0.05).Values are expressed as mean ± standard deviation (SD), and were compared by t-t est.BMI; Body mass index. *n*; no. of sample. *P*-value a: Underweight *vs.* Normal. *P*-value b: Normal *vs*. Obese.

Table 3: Descriptive of females group data (*n* =79)

Variables	BMI class			*P* value[a]	*P* value[b]
	Underweight	Normal	Obese		
N	16 (22.5%)	27 (38%)	28(39.4%)		
Age (Years)	20.50±3.29	19.32±3.27	29.62±16.21	0.21	0.002**
Weight (Kg)	41.10±4.77	52.09±5.40	78.40±10.85	0.000**	0.000**
Height(m)	1.58±.06	1.57±.049	1.58±.05	0.65	0.71
BMI (Kg/m^2)	16.48±1.45	21.06±1.73	31.42±3.86	0.000**	0.000**

** highly Significance (*P*<0.05).Values are expressed as mean ± standard deviation (SD), and were compared by t-t est.BMI; Body mass index. *n*; no. of sample. *P*-value a: Underweight *vs.* Normal. *P*-value b: Normal *vs*. Obese.

Table 4: The distribution of genotypes in all data for SER343SER polymorphisms (*n*=150).

Variables	Frequency%			*P* value [a]	*P* value [b]	*P* value [c]
	TT (*n*=88) 58.7%	TC (*n*=52) 34.7%	CC (*n*=10) 6.7 %			
	Mean ±SD	Mean± SD	Mean±SD			
Age (Years)	21.41±8.85	21.33±8.89	18.80±3.42	0.958	0.36	0.382
Weight (Kg)	65.98±22.33	63.72±22.18	46.40±11.11	0.569	0.000**	0.001**
Height (m)	1.61±.09	1.63±.08	1.60±.10	0.288	0.810	0.424
BMI (Kg/m^2)	25.25±7.79	23.86±7.35	17.83±.81	0.300	0.000**	0.000**

Results are expressed as mean±standard deviation (SD), and were compared by t-test ($P < 0.05$). ** highly Significant difference. *P*-value a: TT *vs.* TC. *P*-value b: TT *vs*. CC. *P*-value c: TC *vs.* CC. *n*; No. of sample.

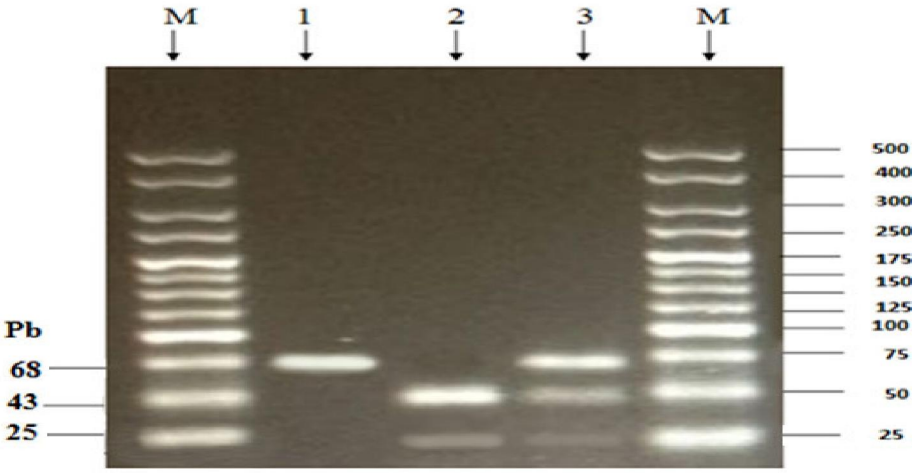

Figure 1: Photograph of a 3% (w/v) agarose gel showing the digested PCR products for SER343SER leptin receptor polymorphism genotyping. Lane M: DNA marker. Lane 1: Homozygous TT; normal genetype that produce one band of size 68 bp. **Lane 2:** Homozygous CC; genetype that produce two bands of size 43 and 25 bp. **Lane 3:** Heterozygous TC; genetype that produce three bands of size 68, 43and 25 bp**.**

Table 5: Males' group data of the distribution of genotypes for the SER343SER polymorphisms (*n*=71).

Variables	Frequency%			*P* - value [a]	*P* - value [b]	*P* - value [c]
	TT (*n*=40) 56.3%	TC(*n*=26) 36.6%	CC(*n*=5) 7%			
	Mean ±SD	Mean± SD	Mean±SD			
Age (Years)	19.10±2.95	18.15±2.42	18.80±3.76	0.17	0.83	0.622
Weight (Kg)	73.70±24.24	67.77±26.52	47.40±15.54	0.35	0.02*	0.04*
Height (m)	1.67±.10	1.66±.087	1.62±.15	0.90	0.32	0.32
BMI(Kg/m^2)	26.52±8.39	24.14±8.39	17.75±3.17	0.26	0.001**	0.009**
Results are expressed as mean±standard deviation (SD), and were compared by t-test ($P < 0.05$). ** highly Significant difference. *P*-value a: TT *vs.* TC. *P*-value b: TT *vs*. CC. *P*-value c: TC *vs.* CC. *n*; No. of sample.						

Table 6:Females'group data of the distribution of genotypes for the SER343SER polymorphisms (*n*=79).

Variables	Frequency%			*P*- value [a]	*P*- value [b]	*P*- value [c]
	TT (*n*=48) 60.8%	TC (*n*=26) 32.9%	CC (*n*=5) 6.3%			
	Mean ±SD	Mean± SD	Mean±SD			
Age (Years)	23.33±11.38	24.50±11.60	18.80±3.49	0.67	0.38	0.29
Weight (Kg)	59.54±18.52	59.67±16.31	45.40±5.81	0.97	0.002**	0.003**
Height (m)	1.56±.04	1.59±.06	1.59±.02	0.38	0.19	0.99
BMI (Kg/m^2)	24.19±7.17	23.57±6.29	17.90±2.16	0.71	0.000**	0.002**
Results are expressed as mean±standard deviation (SD), and were compared by t-test ($P < 0.05$). ** highly Significant difference. *P*-value a: TT *vs.* TC. *P*-value b: TT *vs*. CC. *P*-value c: TC *vs.* CC. *n*; No. of sample.						

Table 7 shows the distribution of genotype and allele frequencies for SER343SER polymorphism in all male and female data. The genotypic frequencies in the underweight group were 45% (n=17) homozygous TT, 37% (n=14) heterozygous TC and 18% (n=7) homozygous CC, the genotypic frequencies in the obese group were 65% (n=37) homozygous TT, 35% (n=20) heterozygous TC and the CC homozygous genotype was undetected in this group, compared to the normal group which were 62% (n= 34) homozygous TT, 33% (n=18) heterozygous TC and 5% (n=3) homozygous CC. In underweight group, the frequency of the T and C alleles were 63% and 37% respectively, and in the obese group, the frequency of the T and C alleles were 82% and 18% respectively, compared to the normal group, the frequency of the T and C alleles were 78% and 22% respectively. In contrast, there were a significant differences in frequencies of alleles between the underweight and the normal group (P= 0.03), but there were no significant differences in frequencies of alleles between the obese and the normal group (P= 0.50).

When comparing the underweight and normal (control) groups results, the frequency of the homozygous TT and heterozygous TC were not significantly difference (*P*= 0.34), but the homozygous TT and CC were significant (*P*=0.03), and heterozygous TC and homozygous CC (*P*=0.14) were not significantly difference. Also, the frequency of dominant model TT and TC+CC genotype were not significantly difference (*P*= 0.10). While the frequency of the T allele was increased in the normal group (78%) compared to the underweight group (63%), the frequency of the C allele was increased in the underweight group (37%) compared to the normal group (22%). So there was an increased frequency of the TT genotype in normal group, and an increased frequency of the TC and CC genotype in underweight group.

When comparing TT and TC genotype the odd ratio was (1.56) (95% CI: 0.63-3.86), and TT and CC genotype the odd ratio was (4.67), which indicated that the odds of underweight is about 4 times higher in CC genotype compared to control (RR=3.60, 95% CI: 1.03-12.57). When comparing TC and CC genotype the odd ratio was (3) (RR=2,33% CI: 0.70-7.82), which indicated that the odds of underweight is about 3 times higher in CC genotype compared to control. TT and TC+CC the odd ratio was (2), which indicated that the odds of underweight is about 2 times higher in TC+CC genotype compared to control (RR=1.45, 95% CI: 0.93-2.25).

When comparing the obese and control groups' results, the frequency of the homozygous TT and the heterozygous TC (*P*= 1), or the homozygous TT and CC (*P*=.11), and the heterozygous TC and the homozygous CC (*P*= 0.10) were not significantly difference. Also, the frequency of dominant model TT and TC+CC genotype were not significantly difference (*P*= 0.72). The frequency of the T allele was increased in the obese group (82%) compared to the normal group (78%), whereas the frequency of the C allele was increased in the normal group (22%) compared to the obese group (18%). There was an increased frequency of the TT and TC genotype in obese group, and an increased frequency of the CC genotype in the normal. When comparing TT and TC genotype the odd ratio was 1.02 (95% CI: 0.45-2.25).

When comparing TT and CC genotype the odd ratio was 0.13 (95% CI: 0.01-2.64), and TC and CC genotype the odd ratio was 0.13 (95% CI: 0.01-2.73), and this indicated the protective effect of CC genotype. When comparing the dominant model between TT and TC+CC the odd ratio was 0.88 (95% CI: 0.41-1.89), this indicated there were no effect and association.

Table 8 shows the distribution of genotype and allele frequencies for SER343SER polymorphism in males group.The genotypic frequencies in the underweight group were 31% (n= 5) homozygous TT, 44% (n= 7) heterozygous TC, and 25% (n= 4) homozygous CC, the genotypic frequencies in the obese group were 68% (n= 19) homozygous TT, 32% (n= 9) heterozygous TC, and the homozygous CC genotype was undetected, compared to the normal group genotyping were 59% (n= 16) homozygous TT, 37 % (n=10) heterozygous TC, and 4% (n= 1) homozygous CC. In underweight group the frequency of the T and C alleles were 53% and 47% respectively, in obese group the frequency of the T and C alleles were 84% and 16% respectively, compared to the normal group the frequency of the T and C alleles were 78% and 22% respectively. In contrast, there were a significant differences in frequencies of alleles between underweight and normal group (*P*= 0.01), but there were no significant differences in frequencies of alleles between obese and normal group (*P*= 0.41).

When comparing the underweight and normal (control) groups results, the frequency of the homozygous TT and heterozygous TC were not significantly difference (*P*= 0.25), the homozygous TT and CC were significant (*P*=0.03), and heterozygous TC and homozygous CC (*P*= 0.31) were not significantly difference. Also, the frequency of dominant model TT and TC+CC genotype were not significantly difference (*P*= 0.07). The frequency of the T allele was increased in normal group (78%) compared to underweight group (53%), whereas the frequency of the C allele was increased in underweight group (47%) compared to the normal group (22%). There was an increased frequency of the TT genotype in normal group, and an increased frequency of the TC and CC genotype in underweight group.

When comparing TT and TC genotype the odd ratio was (2.24), which indicated that the odds of underweight is about 2 times higher in TC genotype compared to control (RR=1.52, 95% CI: 0.77-3.00). When comparing between TT and CC genotype the odd ratio was (12.8), which indicated that the odds of underweight is about 12 fold higher in CC genotype compared to control (RR=7.56, 95% CI: 0.99-57.93).

When comparing between TC and CC genotype the odd ratio was (5.71), indicated that the odds of underweight are about 5 times higher in CC genotype than control (RR=4, 95% CI: 0.53-30-33). TT and TC+CC the odd ratio was (3.2), indicated that the odds of underweight are about 3 times higher in TC+CC genotype than control (RR=1.69, 95% CI: 0.96-2.96).

When comparing the obese and control groups' results, the frequency of the homozygous TT and the heterozygous TC (*P*= 0.62), or the homozygous TT

and CC (*P*=1), and the heterozygous TC and the homozygous CC (*P*=1) were not significantly difference. Also, the frequency of dominant model TT and TC+CC genotype were not significantly difference (*P*= 0.50). The frequency of the T allele was increased in obese group (84%) compared to the normal group (78%), whereas the frequency of the C allele was increased in the normal group (22%) compared to the obese group (16%). There was an increased frequency of the TT genotype in obese, and an increased frequency of the TC and CC genotype in normal group. When comparing TT and TC genotype the odd ratio was 0.76 (95% CI: 0.25-2.32), and TT and CC genotype the odd ratio was 0.28 (95% CI: 0.01-7.40), when comparing TC and CC genotype the odd ratio was 0.37 (95% CI: 0.01-10.18), this indicated there were no effect and association. When comparing the dominant model between TT and TC+CC the odd ratio was (0.69),(95% CI: 0.23-2.08), this indicated there were no effect and association.

Table7: Genotypes and Allele frequencies in all data (*n*= 150).

Genotypes	Frequency% Under weight (n=38)	Normal (n=55)	Obese (n=57)	*P* value[1]	OR[1] (95%CI) / RR[1] (95%CI)	*P* value[2]	OR[2] (95%CI) / RR[2] (95%CI)
TT	45% (17)	62% (n=34)	65% (n=37)	Reference			
TC	37% (n=14)	33% (n=18)	35% (n=20)	0.34	1.56 (0.63-3.86) / 1.30 (0.76-2.24)	1	1.02 (0.46-2.25) / 1.014 (0.61-1.70)
CC	18% (n=7)	5% (n=3)	(n=0)	0.03*	4.67 (1.07-20.35) / 3.60 (1.03-12.57)	0.11	0.13 (0.01-2.64) / 0.14 (0.01-2.67)
				0.14	3 (0.66-13.75) / 2.33 (0.70-7.82)	0.10	0.13 (0.01-2.70) / 0.15 (0.01-2.73)
TC+ CC	55% (n=21)	38% (n=21)	35% (n=20)	0.10	2 (0.86-4.63) / 1.45 (0.93-2.25)	0.72	0.88 (0.41-1.89) / 0.92 (0.56-1.50)
Alleles T	63% (n=48)	78% (n=86)	82% (n=94)	Reference			
C	37% (n=28)	22% (n=24)	18% (n=20)	0.03*	2.09 (1.09-4.00) / 1.69 (1.07-2.68)	0.50	0.76 (0.39-1.48) / 0.80 (0.47-1.37)

Data are presented as number of cases with frequency.1 Between underweight vs. control groups. 2 Between obese vs. control groups. OR: Odds Ratio, RR: Risk Ratio, CI: Confidence Intervals. a: TT *vs*.TC, *P*-value Person Chi-Square test. b: TT*vs*.CC, *P*-value Fisher Exact test. c: TC *vs*.CC, *P*-value Fisher Exact test. d: TT *vs.* TC+CC, *P*-value Person Chi-Square test.

Table 8: Genotypes and Allele frequencies in male group (*n*= 71)

Genotypes	Frequency% Under weight (n=16)	Normal (n=27)	Obese (n=28)	*P* value[1]	OR[1] (95%CI) / RR[1] (95%CI)	*P* value[2]	OR[2] (95%CI) / RR[2] (95%CI)
TT	31% (n=5)	59% (n=16)	68% (n=19)	Reference			
TC	44% (n=7)	37% (n=10)	32% (n=9)	[a]0.25	2.24 (0.56-9.02) / 1.52 (0.77-3.00)	[a]0.62	0.76 (0.25-2.32) / 0.84 (0.40-1.73)
CC	25% (n=4)	4% (n=1)	(n=0)	[b]0.03*	12.8 (1.15 - 142.58) / 7.56 (0.99-57.93)	[b]1	0.28 (0.01-7.40) / 0.3 (0.01-6.91)
				[c]0.31	5.71 (0.52-62.67) / 4 (0.53-30.33)	[c]1	0.37 (0.01-10.18) / 0.4 (0.02-8.78)
TC+ CC	69% (n=11)	41% (n=11)	32% (n=9)	[d]0.07	3.2 (0.87-11.82) / 1.69 (0.96-2.96)	[d]0.50	0.69 (0.23-2.08) / 0.79 (0.39-1.60)
Alleles T	53% (n=17)	78% (n=42)	84% (n=47)	Reference			
C	47% (n=15)	22% (n=12)	16% (n=9)	0.01*	3.09 (1.20-7.95) / 2.11 (1.13-3.92)	0.41	0.67 (0.26-1.75) / 0.72 (0.33-1.58)

Data are presented as number of cases with frequency.1 Between underweight vs. control groups. 2 Between obese vs. control groups. OR: Odds Ratio, RR: Risk Ratio, CI: Confidence Intervals. a: TT *vs*.TC, *P*-value Person Chi-Square test. b: TT*vs*.CC, *P*-value Fisher Exact test. c: TC *vs*.CC, *P*-value Fisher Exact test. d: TT *vs.* TC+CC, *P*-value Person Chi-Square test.

Table 9 shows the distribution of genotype and allele frequencies for SER343SER polymorphism in females group. The underweight group genotyping were 54% (n= 12) homozygous TT, 32% (n=7)

heterozygous TC and 14% (n= 3) homozygous CC, the genotypic frequencies in the obese group were 62% (n=18) homozygous TT, 38% (n=11) heterozygous TC and the CC homozygous genotype was undetected in this group, compared to the normal females group (control) were 64% (n= 18) homozygous TT, 29% (n= 8) heterozygous TC and 7% (n= 2) homozygous CC. In underweight group, the frequency of the T and C alleles were 70% and 30% respectively, in obese group the frequency of the T and C alleles were 81% and 19% respectively, compared to the normal group the frequency of the T and C alleles were 79% and 21% respectively. In contrast, there were no significant differences in frequencies of alleles between underweight and normal group (*P*= 0.35) and also there were no significant differences in frequencies of alleles between obese and normal group (*P*= 0.74).

When comparing the underweight and normal groups results, the frequency of the homozygous TT and heterozygous TC (*P*= 0.67), the homozygous TT and CC (*P*=0.63), and heterozygous TC and homozygous CC (*P*= 1) were not significantly difference. Also, the frequency of dominant model TT and TC+CC genotype were not significantly difference (*P*= 0.48). The frequency of the T allele was increased in the normal group (79%) compared to the underweight group (70%), whereas the frequency of the C allele was increased in underweight group (30%) compared to the normal group (21%). There was an increased frequency of the TT genotype in normal group, and an increased frequency of the TC and CC genotype in underweight group. When comparing TT and TC genotype the odd ratio was 1.31(95% CI: 0.38-4.58), this indicated there were no effect and association, and TT and CC genotype the odd ratio was (2.25), which indicated that the odds of underweight is about 2 times higher in CC genotype compared to control (RR=2, 95% CI: 0.38-10.51). When comparing TC and CC genotype the odd ratio was 1.71(95% CI: 0.22-13.41). TT and TC+CC the odd ratio was 1.5 (95% CI: 0.48-4.69), also there were no effect and association.

When comparing the obese and normal groups results, the frequency of the homozygous TT and heterozygous TC (*P*= 0.57), or the homozygous TT and CC (*P*=1), and heterozygous TC and homozygous CC (*P*= 0.86) were not significantly difference. Also, the frequency of dominant model TT and TC+CC genotype were not significantly difference (*P*= 0.21). There was an increased frequency of the T allele in the obese group (81%) compared to the normal group (79%), and an increased frequency of the C allele in the normal group (21%) compared to the obese group (19%). There was an increased frequency of the TT and CC genotype in the normal group, and an increased frequency of the TC genotype in the obese group. When comparing TT and TC genotype the odd ratio was 1.38 (95% CI: 0.45-4.21) and TT and CC genotype the odd ratio was 0.2 (95% CI: 0.001-4.46), this indicates –nearly- non existence for the effect and association. Also when comparing TC and CC genotype the odd ratio was 1.1(95% CI: 0.37-3.23), when comparing the dominant model between TT and TC+CC the odd ratio was 0.15(95% CI: 0.006-3.23), this indicated there were no effect and association.

Table 9: Genotypes and Allele frequencies in Female group (*n*= 79)

Genotypes	Frequency%			*P* value[1]	OR[1] (95%CI) / RR[1] (95%CI)	*P* value[2]	OR[2] (95%CI) / RR[2] (95%CI)
	Under weight (n=22)	Normal (n=28)	Obese (n=29)				
TT	54% (n=12)	64% (n=18)	62% (n=18)	Reference			
TC	32% (n=7)	29 % (n=8)	38% (n=11)	[a]0.67	1.31 (0.38-4.58) 1.20 (0.53-2.73)	[a]0.57	1.38 (0.45-4.21) 1.23 (0.59-2.59)
CC	14% (n=3)	7 % (n=2)	(n=0)	[b]0.63	2.25 (0.33-15.54) 2 (0.38-10.51)	[b]1	0.2 (0.001-4.46) 0.22 (0.01-4.32)
				[c]1	1.71 (0.22-13.41) 1.5 (0.32-7.14)	[c]0.86	1.1 (0.37-3.23) 1.06 (0.54-2.10)
TC+ CC	46% (n=10)	36% (n=10)	38% (n=11)	[d]0.48	1.5 (0.48-4.69) 1.27 (0.65-2.50)	[d]0.21	0.15 (0.006-3.23) 0.18 (0.01-3.41)
Alleles T	70% (n=31)	79% (n=44)	(n=47) 81%	Reference			
C	30% (n=13)	21% (n=12)	19% (n=11)	0.35	1.54 (0.62-3.81) 1.38 (0.70-2.71)	0.74	0.86 (0.34-2.14) 0.89 (0.43-1.84)

Data are presented as number of cases with frequency.1 Between underweight vs. control groups. 2 Between obese vs. control groups. OR: Odds Ratio, RR: Risk Ratio, CI: Confidence Intervals. a: TT *vs.*TC, *P*-value Person Chi-Square test. b: TT*vs.*CC, *P*-value Fisher Exact test. c: TC *vs.*CC, *P*-value Fisher Exact test. d: TT *vs.* TC+CC, *P*-value Person Chi-Square test.

4- Discussion

In the current study, the distribution of alleles of the leptin receptor gene SER343SER polymorphism in

obese and underweight subjects, compared this with those obtained from normal subjects was studied. The techniques used were PCR analysis of leptin receptor polymorphisms in DNA extracted from blood samples. PCR analysis was carried out for determining genotype. The SER343SER leptin receptor polymorphism investigated in this study results in the substitution of thymine to cytosine (T-C) AGT/AGC in the extracellular domain of the receptor. This replacement gave the amino acid serine again because it has six genetic codes. However, such mutations are extremely rare and are not likely to be responsible for the obesity, because there are many factors that involved and contribute to the appearance of obesity **(Ben Ali *et al*., 2009)**. Data in the literature concerning the association between the SER343SER polymorphism and obesity are controversial among different ethnic population.

Additionally, our results demonstrated that no significant differences were observed with two parameters age and height when screened across genotypes. Significant differences were found with weight and BMI. It is noticed that the subjects carrying the TT genotype had higher BMI (25.25±7.79) than those carrying the CC genotype (17.83±0.81). It is obvious that the T allele (TT and TC) was related to overweight and higher body fat content. In addition, when the subjects were divided into males and females, we found highly significant associations between genotype and BMI in males and females. This significant was observed when the genotype CC was compared with either TT or TC genotypes. Our results were correlated with the finding of **(Mammes *et al*., 2001)**, which detected associations between T allele and overweight and as well as lower response to diet.

In the present study, the results demonstrated that there were a significant differences screened across genotypes in the underweight group compared to normal group between TT and CC genotype (p= 0.03). This suggested that there was an association between the SER(C)343SER(C) polymorphism and underweight. Our finding was in accordance with that obtained by **Okada *et al*., (2009)** who found that there was a significant relationship between Ser343Ser (CC) homozygotes and lower both relative weight for serum lipids and Triglyceride (TG) levels.The genotype and allele frequencies of ser343ser polymorphisms (exon9) were significantly different between underweight and normal group, with the C allele being more frequency in underweight group than in the normal weight group (p=0.03).Also, this study showed no significant differences in genotypic distribution and allele frequencies of the SER343SER polymorphism between obese and normal subjects, which confirmed by **Matsuoka *et al*. (1997)** study.

In this study, results obtained from adult males and females showed that there was an increased frequency of the TC and CC genotype in underweight males and females compared to normal. The CC genotype showed a significant increased in males but not significant in females compared to normal groups. This suggests that there was an association between SER343SER polymorphism and underweight in male but not in females. An increased frequency of the TT genotype in obese males and an increased frequency of the TC genotype in obese females compared to normal was observed, but no significant was detected. Our results were in agreement with previous study conducted by **Mammes *et al*. (2001)** on French women which reported an excess of ser(T)343ser alleles frequency in overweight subjects compared with normal, whatever their origin (TT and TC). The study also reported that the overweight women which carrying the C allele (TC and CC) of this polymorphisms lost more weight in response to low calorie diet than the non carriers **(Mammes *et al*., 2001)**.

In the present study, the codon 343 was found to be CC homozygous in both underweight and normal subjects, while it were not found to be in obese subjects. Our results was agree with **(Matsuoka *et al*., 1997)** study which confirmed that the CC genotype not appear in obese subjects. In contrast, a study in white British population found that the obese men have an increased frequency in TT and CC genotype **(Gotoda *et al*., 1997)**, this was agree with our results in an increased frequency in TT genotype in obese men but was disagreement with **Gotoda *et al*., (1997)** study in an increased frequency in CC genotype in obese men. In our study, the genotype frequencies of Ser(C)343Ser(C) in obese were zero, which was in disagreement with other studies carried out by **Gotoda *et al*. (1997) and Okada *et al*. (2010)**.

Our results of association provided no evidence for a relationship between the SER343SER gene and either obesity or obesity-related phenotypes which was in agreement with that obtained by **Gotoda *et al*. (1997)**. In Obese Spaniards, **Marti *et al*. (2009)** found no significant association between SER343SER polymorphisms and both obesity and serum leptin levels in adult.

The significant association of codon 343 with BMI (P = 0.03) was observed only within the underweight group. However, no possible association of variation at codon 343 with development of obesity was observed, but was with maintenance of low body weight. Previous studies were carried out in other population including Japanese **(Matsuoka *et al*., 1997)**, this studies could not find any significant association of Ser343Ser gene polymorphism, with obesity. When all these findings, taken together, we

found that Ser343Ser coding region were not strongly associated with obesity. The effect of genotypes ser343ser polymorphism on obese may vary from one population to the other as a result of marked differences in the distribution of the alleles in different populations.

Conclusion, based on the results of this study, we found that there was no association between leptin receptor gene SER343SER polymorphisms and obesity in adult's males and females groups; while a significant association in males groups with underweight was observed. Together these results suggest a possible role for leptin receptor gene SER343SER polymorphisms in underweight in males groups and **Recommended** to conduct further studies on females' adults to identify the impact of this gene in Saudi society. Further studies in large samples may be helpful to investigate a more subtle effect of this gene in this serious phenotype. Such studies should also consider possible interactions of this OB-R variant with other genetic polymorphisms.

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Corresponding Author
Dr. Sabah Linjawi,
Biology Department, King Abdulaziz University,
Saudi Arabia Email: slinjawi@kau.edu.sa

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