

TABLE OF CONTENTS

Examination Committee Approval

Dedication

Acknowledgement	i
English Abstract	ii
Arabic Abstract	iii
Table of Contents	iv
List of Figures	vii
List of Tables	viii
List of Symbols and Terminology	ix

Chapter I

Introduction.....	1
-------------------	---

Chapter II

Literature Review	3
2.1 Obesity.....	3
2.2 Obesity in Saudi Arabia.....	4
2.3 The causes of obesity.....	9
2.3.1 Environmental factors.....	9
2.3.2 Genetic factors.....	10
2.4 Leptin.....	11
2.5 Biosynthesis of leptin	12
2.6 Secretion of leptin	14
2.7 Leptin in circulation.....	14
2.8 The leptin receptor.....	15
2.9 Structure of the leptin receptor.....	15
2.10 Location of the leptin receptor.....	19
2.11 Role of leptin in regulation of food intake and body weight	22
2.12 Leptin and obesity.....	24
2.13 Leptin Resistance and obesity.....	26

2.14 Leptin receptor polymorphisms.....	27
2.15 The SER343SER leptin receptor polymorphism.....	29
The aim of study.....	33

Chapter III

Materials and Methods

3.1 List of Materials and Equipments.....	34
3.1.1 Materials and Equipments for DNA Extraction from whole blood.	34
3.1.2 Materials and Equipments for determination of DNA concentration	34
3.1.3 Materials and Equipments for PCR technique.....	35
3.1.4 Materials and Equipments for Purification of PCR products.....	36
3.1.5 Materials and Equipments for Restriction Enzyme	36
3.1.6 Materials and Equipments for Agarose gel electrophoresis	36
3.2 Subjects and study design	37
3.3 Kits.....	40
3.3.1 Genomic DNA Extraction Kit.....	40
3.3.2 Polymerase Chain Reaction (PCR) kit.....	40
3.3.3 Isolate PCR kit.....	41
3.3.4 <i>MluI</i> restriction enzyme kit	41
3.3.5 Primers.....	42
3.3.5.1 Primers Dilution.....	42
3.3.6 Standard.....	42
3.3.7 Buffers.....	42
3.3.7.1 TBE buffer (10X)	42
3.3.7.2 TBE buffer (1X)	43
3.4 Methods.....	43
3.4.1 Anthropometric measurement	43
3.4.2 Extraction of Genomic DNA.....	43
3.4.3 Measurement the Concentration of Genomic DNA	44
3.4.4 Polymerase Chain Reaction	45
3.4.4.1 Amplification for SER343SER gene	45
3.4.4.2 PCR amplification	45

3.4.5 Purification of PCR products	46
3.4.6 Electrophoresis	46
3.4.6.1 Preparation of 3% (w/v) agarose gel	46
3.4.6.2 Preparation of Ethidium Bromide.....	47
3.4.6.3 Agarose gel electrophoresis.....	47
3.4.6.4 Visualization of the DNA band.....	47
3.4.7 Genotyping of rs1805134 SNP in SER343SER gene	48
3.5 Statistical analysis.....	48
3.6 Approval of the ethics committee	49
Chapter IV	
Results	50
4.1 Human subjects.....	50
4.2 Genotyping results.....	50
4.3 All data according to SER343SER polymorphisms.....	57
4.4 Males group data according to SER343SER polymorphisms.....	57
4.5 Females group data according to SER343SER polymorphisms.....	58
4.6 Genotype and allele frequencies of SER343SER polymorphism in all data.....	62
4.7 Genotype and allele frequencies of SER343SER polymorphism in males group.....	67
4.8: Genotypes and Allele frequencies in Female group.....	72
Chapter V	
Discussion	76
List of References	81
List of Electronic References	91
Appendix	92
Summary	104
Arabic Summary	

LIST OF FIGURE

Figure	Page
Figure 2.1: Tertiary structure of leptin	13
Figure2.2: Leptin receptor isoforms , Transmembrane (TM), Mitogen Activate Protein Kinase (MAPK)	17
Figure2.3: The leptin receptor (OB- R) protein and gene structure in human (chromosome1p31).....	18
Figure 2.4: Localization of functional leptin receptors showing the involvement of leptin in peripheral effects.....	21
Figure 2.5: Biologic responses to high versus low leptin levels	25
Figure 2.6: Schematic representation of the Ob-Rb gene (upper panel) and its protein structure (lower panel)	28
Figure2.7:The locations of the SER343SER polymorphisms in exon 9, shown with the published cDNA sequence	31
Figure3.1: Subjects and study design.....	39
Figure 4.1: Photograph of a 3% (w/v) agarose gel showing the result of amplification of human exon 9 in leptin receptor gene by PCR.....	52
Figure 4.2: Photograph of a 3% (w/v) agarose gel showing the digested PCR products for SER343SER leptin receptor polymorphism genotyping....	53
Figure4.3: The frequency distribution of SER343SER genotypes in all data.....	66
Figure4.4: The frequency distribution of SER343SER genotypes in male.....	71
Figure4.5 The frequency distribution of SER343SER genotypes in female.....	75

LIST OF TABLES

Table	Page
2.1: Classification of overweight and obesity in adults according to BMI	5
2.2: The prevalence of obesity ($BMI \geq 30 \text{ kg/m}^2$) according to regions	5
2.3: Comparison of prevalence of overweight (body mass index $25 < BMI < 30$) and obesity (body mass index $\geq 30 \text{ Kg/m}^2$) in Saudi Arabia and four other countries.....	8
2.4: Review the association of SER343SER polymorphism of OB-R gene with obesity for different studies.....	32
4.1 Descriptive of data for all volunteers ($n = 150$).....	54
4.2: Descriptive of males group data ($n = 71$).....	55
4.3: Descriptive of females group data ($n = 79$).....	56
4.4: The distribution of genotypes in all data for SER343SER polymorphisms ($n=150$).....	59
4.5: Males' group data of the distribution of genotypes for the SER343SER polymorphisms ($n=71$).....	60
4.6: Females' group data of the distribution of genotypes for the SER343SER polymorphisms ($n=79$).....	61
4.7: Genotypes and Allele frequencies in all data ($n=150$)	65
4.8: Genotypes and Allele frequencies in male group ($n= 71$).....	70
4.9: Genotypes and Allele frequencies in Female group ($n=79$).....	74

LIST OF SYMBOLS AND TERMINOLOGY

ARC	Arcuate Nucleus
AgRP	Agouti-related peptide
BBB	Blood brain barrier
BMI	Body mass index
bp	Base pairs
C	Cysteine
C-terminal	COOH or Carboxyl-terminal
CCK	Cholecystokinin
cDNA	Complementary DNA
CI	Confidence interval
CART	Cocaine- and amphetamine-regulating transcripts
CRH	Corticotrophin releasing hormone
db/db	Diabetic rat
DNA	Deoxyribonucleic acid
DMH	Dorsomedial hypothalamic nucleus
EDTA	Ethylene diamine tetra acetic acid
FFA	Free fatty acids
IL-6	Interleukin 6

JAK	Janus-Activated kinase
KSA	Kingdom of Saudi Arabia
Kg/m ²	Kilograms/ meter ²
Kb	Kilo base
KDa	Kilo dalton
Lep gene	Leptin gene
LepR	Leptin receptor
MAPK	Mitogen-Activated Protein Kinase
MluI	Micrococcus luteus
mg	Milligram
ml	milliliter
μl	Micro liter
ng/ml	Nanogram/milliliter
NPY	Neuropeptide Y
N-terminal	Amino-terminus or NH ₂ -terminus
Ob	Obese
ob/ob	Obesity mice
Ob gene	Obese gene
ObR	Leptin receptor
OB-Ra	Leptin Receptor Short-form (type a)
OB-Rb	Leptin Receptor Long-form (type b)
Ob-Rc	Leptin receptor type c

Ob-Rd	Leptin receptor type d
Ob-Re	Leptin receptor type e
OR	Odds ratio
PCR	Polymerase Chain Reaction
POMC	Proopiomelanocortin
PPY3 -36	Pancreatic peptide Y3-36
PTP1B	Phosphoryrosine phosphotase 1B
PVN	Paraventricular nuclei
RR	Risk ratio
RFLP	Restriction fragment length polymorphism
rpm	Round per minute
S	Second
STAT	Signal transducers and activators of transcription
SOCS-3	Suppresser of cytokine signaling 3
SNPs	Single nucleotide polymorphisms
SD	Standard deviation Sciences
SPSS	Statistical Package for Social
T	Thiamine
TBE	Tris-base- boric acid- EDTA buffer
TM	Transmembrane domain
TG	Triglycerides
U	Unit

VMH	Ventromedial Hypothalamic Nucleus
WAT	White adipose tissue.
WHO	World Health Organization
χ^2	Chi square
α	Alpha
α MSH	α melanocyte stimulating hormone.

Chapter I

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 gains are major health problem which increased among Saudi population over the past thirty years. The total obesity reached in national study on Saudis adults in 2005 to 35.5%. (Al-Nozha *et al.*, 2005; Al-Sultan *et al.*, 2006; Enriori *et al.*, 2006 ; Marti1 *et al.*, 2009; Bahathiq, 2010).

According to World Health Organization (WHO) statistics, in 2008 39.1% females and 28.6% males were obese in Saudi Arabia .This value is higher than that reported in the British, Australian, Americans and Italian populations. It is rising and alarming, especially among females (Al-Nozha *et al.*, 2005; Bahathiq, 2010). So health authorities must take the necessary measures to fight obesity responsible for many health problems and start health education programs about the health risks of obesity on the individuals. Scientific researches are also needed to identify the causes of obesity, its treatment how to prevent it, improve eating habits and levels of physical activity to the community.

Recently, several studies have been directed to investigate the variations in the level of leptin (obesity hormone), derived from adipose tissue in an attempt to

determine if it can be used as a marker of obesity .Since leptin works to reduce appetite and increase energy expenditure by binding to its receptor, Studies have focused on the leptin receptor polymorphisms because they 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 SER 343 SER, which resulted by silent mutation gene across the replacement of nitrogen base 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 (TCT- TCC- TCA- TCG- AGT - AGC).

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