Islamic University-Gaza
Deanery of Higher Education
Faculty of Science
Master of Biological Sciences
Medical Technology

الجامعة الإسلامية-غزة
عمادة الدراسات العليا
كلية العلوم
ماجستير العلوم الحياتية
تحاليل طبية

Insulin, thyroid stimulating hormone and lipid levels among obese adult males in Gaza Governorate.

Prepared by

Maher Abdel - Rahman Al-Jedi

Supervisors

Dr. Mohammad E. Shubair
Professor of Medical Technology
Faculty of Science

Dr. Maged Yassin
Professor of Human Physiology
Faculty of Medicine

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Biological Sciences/Medical Technology

1432هـ - 2011م

بسم الله الرحمن الرحيم

قال تعالى

" وَمَا أُوتِيتُم مِّنَ الْعِلْمِ إِلَّا قَلِيلًا "

"وَقُل رَّبِّ زِدْنِي عِلْمًا"

صدق الله العظيم

Declaration

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains material neither previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree of the university of other institute, except where due a acknowledgment has been made in the text.

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Insulin, thyroid stimulating hormone and lipid levels among obese adult males in Gaza Governorate.

Abstract

Objective: This study aimed to asses the levels of insulin, thyroid stimulating hormone (TSH), glucose and lipid profile among obese adult males in Gaza Governorate. The relation between such parameters and obesity grades as well as between insulin, TSH and the other parameters were established.

Methodology: The study sample comprised 82 obese males aged between 20-40 years. The control group consisted of 82 non-obese individuals matched cases with age. TSH and insulin levels were determined using ELISA technique while lipid profile and glucose assayed by enzymatic methods. Demographic and personal data were collected using questionnaire interview. Data were analyzed using SPSS program.

Results: Obesity was significantly related to history of hyperlipidemia, inappropriate weight gain and family history ($p<0.05$). Getting fatigue quickly, muscle weakness and consumption of marine food, and large quantities of carbohydrates and lipids-rich food were significantly related to obesity. Walking regularly and having active job were also related to obesity. The mean levels of insulin and glucose were significantly increased in cases compared to controls (mean=13.0±13.0 µIU/mL and 91.0±20.2 mg/dl Vs 6.9±8.0 µIU/mL and 83.7±11.2 mg/dl, P=0.000 and 0.005, respectively). In contrast, the mean levels of TSH were lower in cases compared to controls (mean = 1.41±1.23 µIU/mL Vs 1.93±0.98 µIU/mL, P=0.003). The mean levels of total cholesterol, triglycerides and LDL were increased in cases compared to controls (mean=187.3±38.5 mg/dl,139.2±63.7mg/dl and119.4±34.8 mg/dl Vs174.9±43.5 mg/dl, 98.9±68.6 mg/dl and 107.9±42.4 mg/dl, respectively), whereas the mean level of HDL was significantly decreased in cases compared to controls (40.1±10.9 mg/dl Vs 47.7±9.6 mg/dl). There were no significant relations between the studied parameters including insulin, TSH, glucose, total cholesterol, triglyceride, LDL and HDL, and different grades of obesity. The increases in the mean levels of insulin throughout the three categories of< 9, 9-18 and >18 µIU/mL were significantly associated with

increasing glucose levels (F=5.619, P=0.005). On the other hand, the relation of insulin with other parameters was not significant (P>0.05). There was no significant relation between TSH and other parameters (P>0.05). Insulin level showed strong positive correlation with glucose level (r=0.470). This correlation was significant (P=0.000). TSH level showed relatively weak negative correlations with total cholesterol and LDL levels (r=-0.233, r=-0.227, respectively). Such correlations were significant with P=0.036 and P=0.041, respectively.

Recommendations: We recommend that obese individuals must follow diet and do exercise to reduce weight in order to avoid dangerous diseases. Insulin test is a very important test for obese individuals; this test may predict if the obese person is pre-diabetic or not and frequent monitoring of lipid profile is necessary for obese individuals.

Key words: Insulin, thyroid stimulating hormone, obesity, lipids , Gaza Governorate

قياس مستوي هرمون الأنسولين،محفز الغدة الدرقية ، ومستويات الدهون بين مجموعة من الذكور السمان البالغين في محافظة غزة.

ملخص الدراسة

هدف الدراسة:هدفت الدراسة لقياس مستوي هرمون الأنسولين، محفز الغدة الدرقية ، الجلوكوز ومستويات الدهون بين مجموعة من الذكور السمان البالغين في محافظة غزة.تم إنشاء علاقة بين المتغيرات السابقة ودرجات السمنة المختلفة كما وتم إنشاء علاقات بين هرموني الأنسولين ومحفز الغدة الدرقية وباقي المتغيرات الاخري.

الطرق والأدوات: تكونت عينة الدراسة من 82فرد من الذكور السمان الذين تتراوح أعمارهم من20-40 سنة. أما المجموعة الضابطة فقد تكونت من82 فرد من الذكور ذوي الوزن الطبيعي مع وجود توازن في الأعمار بين مجموعتي الدراسة . تم تحديد مستوي هرموني الأنسولين ومحفز الغدة الدرقية باستخدام تقنية ELISAبينما تم قياس مستوي السكر ومستويات الدهون باستخدام الطرق الإنزيمية. تم تحليل البيانات والنتائج التي تم الحصول عليها باستخدام البرنامج الإحصائي SPSS.

النتائج : كان هناك علاقة ذات دلالة إحصائية بين السمنة وكل من المتغيرات التالية (ارتفاع مستوي الدهون في الدم في الماضي، الزيادة اللافتة للنظر في الوزن، والتاريخ المرضي في العائلة،الشعور السريع بالتعب،آلام العضلات،تناول كميات كافية من المأكولات البحرية،تناول كميات كبيرة من الأطعمة الغنية بالدهون والكربوهيدرات،ممارسة رياضة المشي بشكل منتظم،العمل بوظيفة تتطلب الحركة). وقد أظهرت النتائج أن متوسط مستوي هرمون الأنسولين والجلوكوز كان أعلى في العينات المرضية (السمان) مقارنة بالعينة الضابطة ،وقد كانت النتائج ذات دلالة إحصائية. من ناحية أخري كان متوسط مستوي هرمون محفز الغدة الدرقية في العينة المرضية (السمان) اقل منها في العينة الضابطة وقد كانت النتائج ذات دلالة إحصائية. كانت نتيجة متوسط مستوي كل من الكولسترول،الدهون الثلاثية والبروتين ألدهني منخفض الكثافة أعلى في العينة المرضية (السمان) منها في العينة الضابطة، بينما كان متوسط مستوي البروتين ألدهني عالي الكثافة اقل في العينة المرضية منه في العينة الضابطة. لم تكن هناك أي علاقة ذات دلالة إحصائية بين المتغيرات الكيميائية المدروسة (الأنسولين،محفز الغدة الدرقية،الجلوكوز ،الكولسترول ،الدهون الثلاثية،البروتين ألدهني عالي الكثافة والبروتين ألدهني منخفض الكثافة) والمستويات المختلفة للسمنة. كان معدل الزيادة في متوسط مستوي هرمون الأنسولين (عند أفراد العينة المرضية) مرتبط بزيادة متوسط مستوي الجلوكوز وقد أظهرت النتائج دلالة إحصائية واضحة، بينما كانت علاقة هرمون الأنسولين مع المتغيرات الكيميائية ألاخري ليست ذات دلالة إحصائية. لم يكن هناك أي علاقة ذات دلالة إحصائية بين المتغيرات الكيميائية المدروسة والمستويات المختلفة لهرمون محفز الغدة الدرقية عند أفراد العينة المرضية (السمان). ظهرت علاقة طردية قوية ذات دلالة إحصائية عند أفراد العينة المرضية (السمان) بين مستوي الأنسولين والجلوكوز، من ناحية أخري كانت العلاقة بين محفز الغدة الدرقية وكل من الكولسترول والبروتين ألدهني منخفض الكثافة علاقة عكسية وضعيفة نسبيا.

التوصيات : ونوصي مرضي البدانة بإتباع نظام غذائي صحي وممارسة الرياضة من اجل تخفيف أوزانهم

وذلك لتجنب الكثير من الأمراض الخطيرة ،كما ويعتبر فحص الأنسولين ضروري لتحديد مدي الاستعداد للإصابة بمرض السكر،كذلك نوصي مرضي البدانة بالمتابعة الدورية لمستويات الدهون في الدم.

الكلمات المفتاحية: الأنسولين ،محفز الغدة الدرقية،السمنة،الدهون ،محافظة غزة.

Dedication

To my father soul

To my dear mother

To my brothers, my sisters

To my wife, who encourages me in my study

To my daughters

Mariam, Mira, Malak

To all of them I dedicate this work

Acknowledgment

I would like to express my sincere thanks to all people who directly or indirectly have contributed to this work. In particular I am greatly and deeply indebted to my supervisors **Dr. Mohammad shubair** professor of medical technology, and **Dr. Majed yassin**, professor of human physiology who stood with me step by step. With their support ,devise, patience, special insights and encouragements, this research finally came to light and realized. Special appreciation should be extended to all staff at the department of the biological science master program, and all staff at the medical technology department in the Islamic university for their support and help.

Special thanks to the Islamic university clinic, especially Dr. Alli Al- Zinaty, for his help and facilities during sample collection.

Special thanks for the dearest person to me my mother, and my beloved wife Engineer Ghada, for their support and encouragements.

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Abbreviations

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Adenosine triphosphate	**ATP**
Agouti-related peptide	**AgRP**
B-cell lymphoma 1	**Bcl-2**
Body mass index	**BMI**
Cholecystokinin	**CCK**
Cholesterol esterase	**CHE**
Cholesterol oxidase	**CHOD**
Coronary heart disease	**CHD**
Cyclic adenosine monophosphate	**cAMP**
Diiodotyrosine	**T2**
Diiodotyrosine	**DIT**
Enzyme linked immunosorbent assay	**ELISA**
Fatty acid synthase	**FAs**
Glucose oxidase	**GOD**
Glucose transporters 4	**GLUT4**
Glycerol kinase	**GK**
Glycerol phosphate oxidase	**GPO**
High density lipoprotein cholesterol	**HDL-C**
Hormone sensitive lipase	**HSL**
Hypothalamic-pituitary-thyroid	**HPT**
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Lipoproteinlipase	**LPL**
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Thyroid releasing hormone	**TRH**

Thyroid stimulating hormone	**TSH**
Thyroxine	**T4**
Thyroxine binding globulin	**TBG**
Total cholesterol	**TC**
Triacylglycerol	**TAG**
Triiodothyronine	**T3**
Uncoupling protein	**UCP**
Very low density lipoprotein-cholesterol	**VLDL-C**

Chapter 1

Introduction

1. Introduction

1.1 Overview

Obesity can lead to several diseases that impact negatively on quality of life, morbidity and mortality outcomes in large population groups (1). Obesity is associated with a wide variety of co-morbidities, some of which may lead to disability or death (2). Approximately 1.2 billion people in the world are overweight and at least 300 million of them are obese. According to the World Health Organization (WHO), obesity is one of the 10 most preventable health risks, and the frequency of obesity around the world is 25 percent where 10 percent of those, morbid obeses, have body mass index (BMI) >39 (3). Thyroid hormones affect in a myriad of biological processes such as development, growth and metabolic control, as it influence all major metabolic pathways (4).

Their most obvious and well-known action is an increase in basal energy expenditure obtained acting on protein, carbohydrate and lipid metabolism. With specific regard to lipid metabolism, thyroid hormones affect synthesis, mobilization and degradation of lipids, although degradation is influenced more than synthesis (5). The association of obesity with type 2 diabetes has been recognized for decades, and the major basis for this link is the ability of obesity to engender insulin resistance (6).

There are also grounds for considering the related possibility that insulin resistance and hyperinsulinemia, in addition to being caused by obesity, can contribute to the development of obesity (7). The relationship between obesity and insulin resistance is seen across all ethnic groups and is evident across the full range of body weights (8). Central (intra-abdominal) depots of fat are much more strongly linked to insulin resistance, type 2 diabetes, and cardiovascular disease than are peripheral (subcutaneous) fat depots (9). It is possible that an unknown common factor, either genetic or environmental, produces both insulin resistance and the central pattern of regional adiposity, and that central obesity does not actually cause insulin resistance.

Alternatively, some biochemical feature of intra-abdominal adipocytes may directly influence systemic insulin sensitivity (7).

The relationship between obesity, lipid profile, insulin levels and thyroid dysfunction is a concern for researchers, and studies are being carried out to link up these variables. Lipid and thyroid profiles are the most common investigations called for in obese subjects by clinicians (10,11). Obesity is associated with derangements in the lipid profile, which further increases the risk of coronary heart disease, diabetes mellitus, stroke and certain cancers.
In some studies, higher total cholesterol (TC) , Triacylglycerol (TAG) , low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) levels were observed in obese subjects as compared to controls except high density lipoprotein cholesterol (HDL-C) , which was significantly lower in obese subjects (12). When the TSH levels were correlated among the obese subjects with grade I and grade II obesity according to BMI values, a significant difference in TSH levels were observed, highlighting the variation in TSH levels depending on the extent of obesity (12).

Other previous studies, reported that an increase in BMI was associated with an increase in TG, and a decrease in HDL-C levels (13,14, 15). Early studies also revealed an association between body mass index (BMI) and TSH levels, showing varying TSH levels depending on the degree of obesity from mild to severe (16). As the lipid profile is deranged with higher BMI, it impairs resistance to TSH in peripheral tissue and further aggravating the thyroid problem (12). Hypothyroidism is linked to obesity, and so there must be some link between the thyroid profile and the lipid profile, as derangements of lipid profile are observed in obesity (14,15).

In the European population, a positive correlation has been established between obesity (BMI > 30 kg/m2) and TSH level (17). In a study on 226 euthyroid obese or over weight female patients there was significant positive correlation between serum TSH and fasting plasma insulin (18). The association between normal range thyroid function and BMI and dyslipidemia has been the subject of much debate. A study showed a positive correlation

between BMI and serum TSH, a negative correlation between BMI and serum free T4, and no association between BMI and serum free T3 (19). Other study in obese women without complications also showed a positive correlation between normal range serum TSH and BMI (20).

However, investigation of another cohort 401 euthyroid subjects showed no significant relationship between BMI and either serum TSH concentration or free T4 (21). These findings suggest that thyroid function within the normal range may be associated with BMI, but a definitive relationship is not clear at this point(22). While some studies have found that thyroid disorders may lead to obesity, recent studies on obese children showed that it is the obesity that may cause the disorder. In a recent study dealt with the alterations in thyroid function and structure these alterations are common in obese children. An association between body mass index and thyroid hormone levels was found. This suggests that fat excess may have a role in thyroid tissue modification and thyroid function has been shown to return to normal after weight loss (23,24).

1.2 Objectives

The overall objective is to asses insulin, thyroid stimulating hormone, glucose and lipid levels among obese adult males in Gaza Governorate.

The specific objectives are:

1. To asses risk factors of obesity

2. To determine insulin, glucose and TSH levels among obese adults in comparison to non obese individuals.

3. To determine total cholesterol, Triacylglycerol, high-density lipoprotein cholesterol, and low density lipoprotein cholesterol among the obese and non obese individuals.

4. To evaluate the relationship between obesity grades and insulin, TSH, glucose and lipid profile.

5. To find out the relation between insulin, TSH, and the other studied parameters

1.3 Significance

1. Obese subjects may present with abnormal thyroid function, but reported data in Gaza governorate are scarce.

2. Examination of TSH is required in obese subjects in an attempt to understand the underlying cause of obesity.

3. To make obese subjects aware of risk factors of obesity.

4. Some obese people have insulin resistance (pre-diabetic) but don't even know, by maintaining an appropriate weight and active life style, many individuals are able to reduce their chances of becoming diabetic patient.

Chapter 2

Literature Review

2. Literature review

2.1 Obesity

2.1.1 Definition

Obesity is defined as an excess accumulation of body fat and is known to increase the risk of various pathological states such as hypertension, dyslipidemia and coronary heart disease (25). Obesity, especially abdominal obesity is a very important risk factor of cardiovascular diseases and some types of cancer. It is also conducive to the development of metabolic and rheumatic diseases, diseases of the liver and biliary ducts, as well as respiratory diseases (26). Obesity is the consequence of an overall positive energy balance maintained over time, that is, the metabolizable energy intake exceeds the energy expenditure for basal metabolic requirements, thermoregulation, physical activity, and growth (27).

2.1.2 Chemical factors affecting obesity

There is a number of naturally occurring chemicals involved in monitoring energy levels, energy expenditure, or current energy supplies. Among the factors that relay this information are both the neurotransmitters including serotonin, dopamine, epinephrine, and histamine, as well as other factors that relay messages about food intake and energy levels such as leptin, cholecystokinin (CCK), neuropeptide Y(NPY), ghrelin, agouti-related peptide (AgRP), and adiponectin (28). These chemicals are some of the messengers that constantly relay information regarding the body's levels of available and stored energy to the brain, and there is significant redundancy in their effects. For example, cholecystokinin, adiponectin, leptin, serotonin, and histamine all suppress appetite, whereas ghrelin, agouti related protein, and neuropeptide Y all increase appetite (28) .

2.1.3 Causes of obesity

1- Genetics: The role of a genetic predisposition to obesity has long been recognized to affect both terms (intake and expenditure) of the energy balance equation (29). Genes may influence afferent and efferent signals as

well as central mechanisms involved in body-weight regulation (30,31). An update of the genetics of human obesity revealed that the numbers of genes or markers that have been linked with human obesity are increasing rapidly and now approaching 200 genes (32,33). The possible physiological mechanisms through which a genetic susceptibility may be operating include low resting metabolic rate, low rates of oxidation, low fat-free mass and altered food intake, as well as other factors related to macronutrient utilization, energy expenditure or the hormonal profile, including insulin sensitivity (34,25). The occurrence of genes or mutations responsible for the susceptibility of some individuals or groups of individuals to gain weight in the presence of an energy-dense diet or a reduced daily physical activity is being currently investigated (35,36).

2- Physical inactivity: Most available evidence suggests that a lower activity-related energy expenditure is an important contributor to the increasing prevalence of obesity, although a blunted response to food intake and reductions in resting energy expenditure may have an impact on weight gain (37,38). Furthermore, studies have often found an associations between leisure-time physical activity (inverse) or total amount of time spent sitting down (direct) and BMI (39), while a low participation in sports activities, a lack of interest in taking exercise and a high number of hours spent sitting down at work are statistically significant predictors of obesity (40).

3- Overeating: Overeating is a relative term. It refers to the consumption of an energy intake that is inappropriately large for a given energy expenditure, thus, leading to obesity, Physical inactivity compounds the effects of high-fat, energy-dense diets, causing positive energy balance (41).

4- Diseases: Diseases such as hypothyroidism, insulin resistance, polycystic ovary syndrome and Cushing's syndrome, are also contributors to obesity (42).

5- Psychological factors: A variety of psychosocial factors contribute to the development of obesity and to the difficulty of losing weight. For some people,

emotions influence eating habits. Many people eat excessively in response to emotions such as boredom, sadness, stress or anger (43) .

6- Medications : Medications are documented to increase weight gain include antipsychotics (phenothiazines, butyrophenones), antidepressants, antiepileptics, insulin and some oral hypoglycemics. Whereas most of these medications contribute modestly to obesity, the large doses of steroids sometimes used to treat autoimmune diseases or used as contraceptive can cause true obesity (44).

2.1.4 Classification and assessment of obesity

The initial step in evaluation of obesity is the calculation of BMI. To measure BMI, one begins by weighing the patient in underclothes and without shoes. Height as well measured without shoes. BMI is calculated by dividing weight (in kilograms), by square height (in meters). BMI has replaced percentage ideal body weight as a criterion for assessing obesity for several reasons. BMI correlates significantly with body fat, morbidity, and mortality, and it can be calculated quickly and easily. Furthermore, recommendations for treatment of obesity are based on BMI. A BMI of 25 kg/m2 is the generally accepted threshold for identifying a patient at higher risk for obesity-related diseases, most notably type 2 diabetes, hypertension, and cardiovascular disease (45). Risk of death begins to increase at a BMI of 23 kg/m2 when compared with the lowest risk group (BMI, 19.0 to 21.9 kg/m2) (46).

Medical risk rises progressively with increasing degrees of obesity beginning with overweight, defined by BMI between 25.0 and 29.9 kg/m2, through class I obesity (BMI, 30.0 to 34.9 kg/m2), class II obesity (BMI, 35.0 to 39.9 kg/m2), and class III or morbid obesity (BMI > 40 kg/m2) (47). This classification system of obesity by BMI was developed by the World Health Organization Obesity Task Force and has been adopted by researchers on the identification, evaluation, and treatment of overweight and obesity in adults (48,49). Waist circumference is an important measure of obesity risk. The measuring tape should be snug but not compressing the skin and held parallel to the floor. The measurement is made at normal respiration (49). A high-risk

waist circumference is accepted to be 35 inches (88 cm) or greater for women and 40 inches (102 cm) or greater for men. Waist circumference is a practical indicator of visceral abdominal fat. Evidence suggests that abdominal fat carries a higher health risk than peripheral fat, and that the visceral fat component correlates the most strongly with increased risk (49). Some epidemiological studies have found the waist-to-hip ratio to correlate with increased risk for diabetes, coronary heart disease(CHD), and hypertension (49), however, this measure is not established as an independent risk factor. Waist circumference also has been found to be a superior indicator of abdominal fat distribution (49).

The truncal fat distribution indicated by an increased waist circumference correlates with the hypertrophic form of obesity. Hypercellular obesity, which is characterized by an increased total number of fat cells, typically affects patients with a BMI less than 40 kg/m2 but may be a lower risk form of disease. In hypertrophic obesity, existing fat cells enlarge and produce proteins and metabolites` involved in the pathophysiology of obesity (50). These proteins include lipoprotein lipase, which contributes to hydrolysis of the triglycerides of very-low-density lipoproteins (VLDL) and chylomicrons, and cytokines (tumor necrotizing factor-and interleukin-6), as well as resistin (51). The hypertrophied fat cell also produces leptin hormone. Hypertrophic obesity correlates with metabolic complications of obesity, including impaired glucose tolerance, adverse lipid profile, hypertension, and CHD (50). Also, age of onset helps to distinguish hypercellular from hypertrophic obesity, because the hypercellular form often begins in childhood, whereas hypertrophic obesity often begins in adulthood (52).

2.2 Insulin

2.2.1 Definition and structure

Insulin is a small protein; human insulin has a molecular weight of 5808. It is composed of two amino acid chains, connected to each other by disulfide linkages (figure 2.1.A) . When the two amino acid chains are split apart, the functional activity of the insulin molecule is lost. Insulin is synthesized in the

pancreatic beta cells by the usual cell machinery for protein synthesis beginning with the translation of the insulin RNA by ribosomes attached to the endoplasmic reticulum to form an insulin preprohormone. This initial preprohormone is cleaved in the endoplasmic reticulum to form proinsulin. Most of this is further cleaved in the Golgi apparatus to form insulin and peptide fragments before being packaged in the secretory granules (figure 2.1.B) (53).

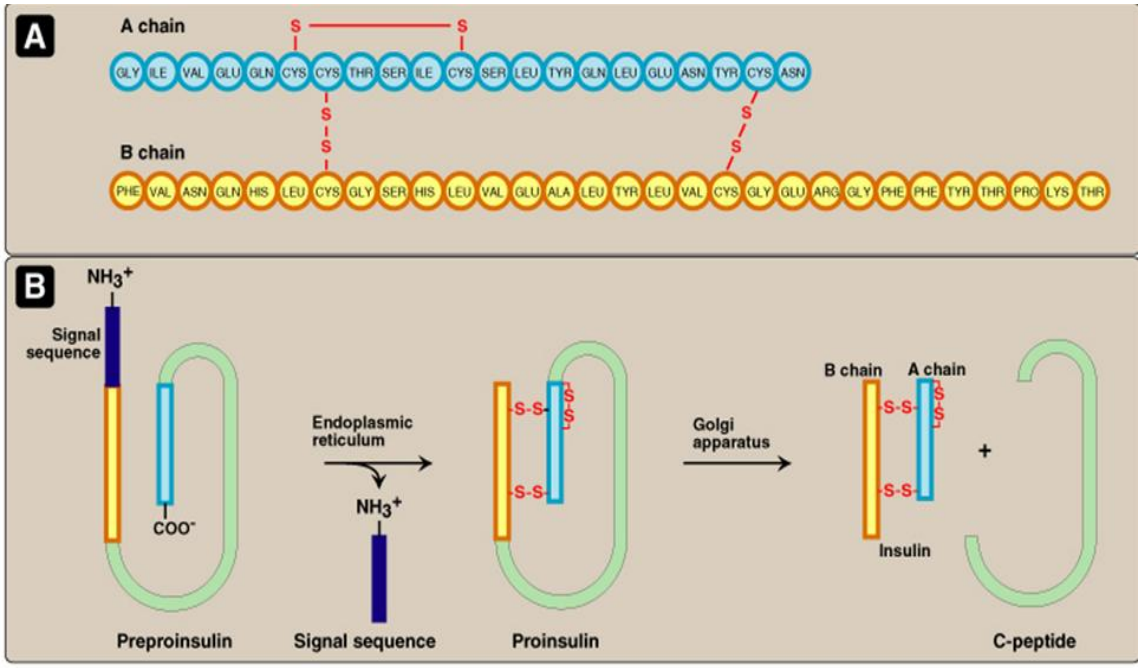

Figure 2.1 A. Structure of insulin. B. Formation of human insulin from preproinsulin

2.2.2 Secretion

Insulin, a hormone secreted by the beta cells of the pancreas, plays a predominant role in the lipogenic process (54).

2.2.3 Insulin receptors

The insulin receptor is a large transmembrane glycoprotein found in insulin sensitive target cells (liver, muscle, and fat) (55). It comprises two extracellular R-subunits that contain the insulin-binding domain and two membrane spanning subunits that contain a ligand activated tyrosine kinase, which will be referred to as the insulin receptor tyrosine kinase . Insulin acts

by binding to the extracellular domain of the insulin receptor, thus inducing autophosphorylation and activation of the insulin receptor tyrosine kinase. A cascade of signaling events is initiated leading to increased tyrosine phosphorylation of multiple intracellular substrates, including the insulin receptor substrates 1 and 2 , and the activation of second messenger systems such as phosphatidylinositol 3-kinase (56). These pathways act to trigger the translocation of glucose transporters 4 (GLUT4) to the cell surface (57). GLUT4 is one of a family of membrane proteins responsible for glucose uptake in mammalian cells and is the major isoform responsive to insulin stimulation (figure.2.2) (58**) .**

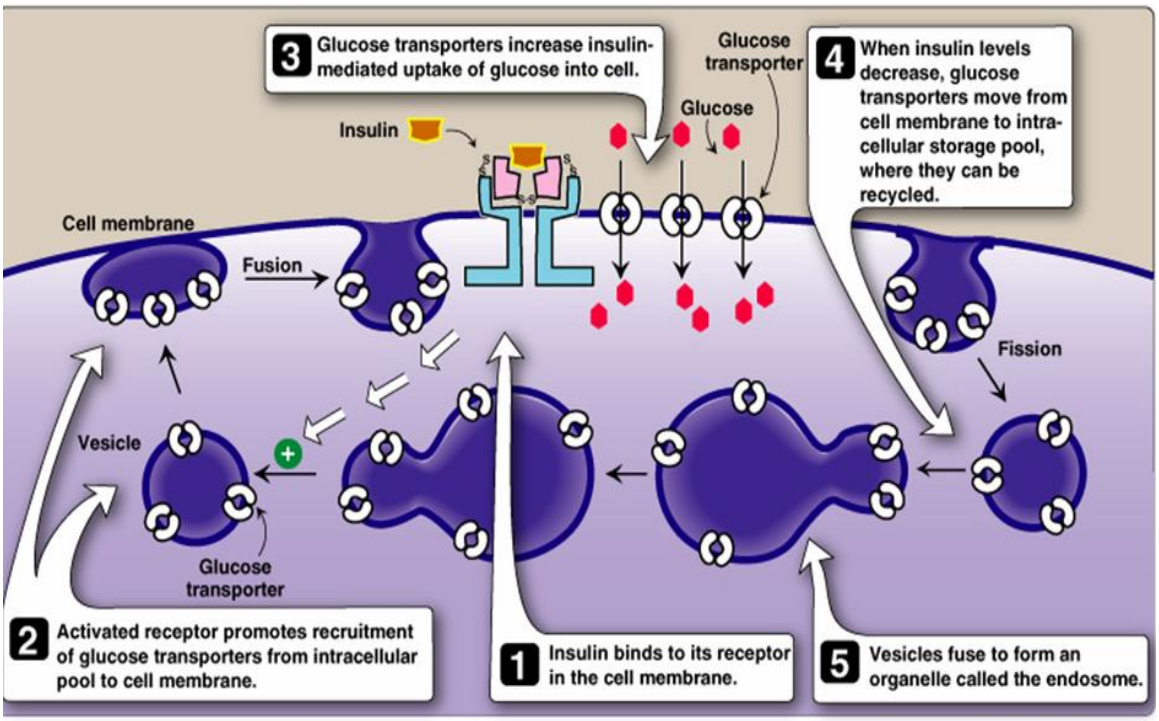

Figure 2.2 Insulin causes cells to recruit transporters from intracellular stores.

2.2.4 Increased blood glucose stimulates insulin secretion

At the normal fasting level of blood glucose of 70 to 110 mg/dl, the rate of insulin secretion is minimal on the order of 25 ng/min/kg of body weight, a level that has only slight physiologic activity. If the blood glucose concentration is suddenly increased to level two to three times normal and kept at this high level thereafter, insulin secretion is increased markedly in two stages **:**

1. Plasma insulin concentration increases almost 10-fold within 3 to 5 minuets after the acute elevation of blood glucose; this results from immediate dumping of preformed insulin from the beta cells of the islet of langerhans. However the initial high rate of secretion is not maintained; instead, the insulin concentration decreases about halfway back toward normal in another 5 to 10 minutes.

2. Beginning at about 15 minuets, insulin secretion rises a second time and reaches anew plateau in 2 to 3 hours, this time usually at the rate even greater than that in the initial phase. This secretion results both from additional release of preformed insulin and from activation of the enzyme system that synthesizes and releases new insulin from the cells (53).

2.2.5 Mechanism of action of insulin

The net effect of insulin is to enhance storage and block mobilization and oxidation of fatty acids. Insulin exerts its effect by stimulating lipoprotienlipase (LPL) formation, so that circulating triglycerides are hydrolyzed and free fatty acids can enter the adipocyte. Insulin is also required for the transport of glucose, which is needed for re-esterification of the triglycerides once inside the adipocyte. Finally, the conversion of glucose to fatty acids is accomplished by insulin's activation of several enzymes (54).

Lipolysis is the chemical decomposition and release of fat from adipose tissue. This process predominates over lipogenesis when additional energy is required. The triglycerides within the adipocyte are acted upon by a multi-enzyme complex called hormone sensitive lipase (HSL), which hydrolyzes the triglyceride into free fatty acids and glycerol. These lipases act consecutively on triglycerides, diglycerides, and monoglycerides. Insulin reduces mobilization of fatty acids from adipose tissue by inhibiting triglyceride lipase. The mechanism of this inhibition may be through a decrease in cyclic AMP which in turn results in an inhibition of cyclic-AMP-dependent protein kinase (54).

2.2.6 Insulin resistance and obesity

The term “insulin resistance” usually connotes resistance to the effects of insulin on glucose uptake, metabolism, or storage. Insulin resistance in obesity and type 2 diabetes is manifested by decreased insulin-stimulated glucose transport and metabolism in adipocytes and skeletal muscle and by impaired suppression of hepatic glucose output (59).

Insulin resistance is traditionally assessed by insulin’s ability to promote normal glucose metabolism. Insulin’s action on lipid metabolism is analogous to its role in glucose metabolism, ie, promoting anabolism and inhibiting catabolism. Specifically, insulin upregulates LPL and stimulates gene expression of intracellular lipogenic enzymes, such as acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS)(60). In addition, insulin inhibits adipocyte HSL through inhibition of its phosphorylation (61) .
In the insulin-resistant state, the responses of both LPL and HSL to insulin are blunted. Thus, with insulin resistance, inefficient trapping of dietary energy occurs both because of decreased LPL-mediated lipolysis of chylomicron-TAG and ineffective inhibition of HSL-mediated lipolysis in adipose tissue (62).

In obesity the fat cells, perhaps because they are already overloaded with TAG, fail in their normal role of protecting other tissues from the daily influx of dietary fatty acids. The increased flux of fatty acids (both as non-esterified and TAG) in the circulation has acute adverse effects on insulin sensitivity, but also leads in the longer term to accumulation of (TAG) in glucose-metabolizing tissues such as skeletal muscle, liver and the pancreatic β-cell (63) . Accumulation of TAG in these tissues, by some unknown mechanism, but probably involving local TAG hydrolysis and availability of fatty acids or fatty acyl-CoA, leads to an impairment of the normal sensitivity of glucose metabolism to insulin (or, in the case of the β-cell, to an impairment of insulin secretion in response to glucose) (63).

2.3 Thyroid stimulating hormone (TSH)

2.3.1 TSH overview

TSH is glycoprotein hormone produced in the thyrotrophs of the anterior pituitary gland. Its synthesis and secretion are stimulated by thyrotropin releasing hormone(TRH) and inhibited by thyroid hormone in a classic endocrine negative feedback loop (64).

2.3.2 TSH Structure

Human TSH consists of two noncovalently linked subunits, α -subunit (92 amino cids; common for other human glycoprotein hormones) and TSH β-subunit. The primary structures of TSH subunits are species specific. Human TSH, for example, differs from bovine TSH by 28 amino acids in the α-subunit and by 12 amino acids in the β-subunit. The coding sequence of the TSH β-subunit gene predicts a 118-amino acid protein (65). However, β-subunit of TSH isolated from cadaver pituitary is composed of 112 amino acids (66), most likely due to proteolytic cleavage during purification (67). TSH, similar to other glycoprotein hormones, is a glycosylated protein. The carbohydrate chains constitute 15-25% of its weight and include three asparagine linked carbohydrate chains (68).

2.3.3 TSH function and physiological role

TSH controls thyroid function upon its interaction with the G protein-coupled TSH receptor (69,70 ,71,72). TSH binding to its receptor on thyroid cells leads to the stimulation of second messenger pathways involving predominantly cyclic adenosine monophosphate (cAMP) and, in high concentrations, inositol 1,4,5-triphosphate and diacylglycerol, ultimately resulting in the modulation of thyroidal gene expression (73).

Physiological roles of TSH include the stimulation of differentiated thyroid functions, such as iodine uptake and organification, the release of thyroid hormone from the gland, and promotion of thyroid growth (65). It also acts as a thyrocyte survival factor and protects the cells from apoptosis (74), via

regulation of p53 and the bcl-2 gene family (75,76). A further interesting finding is that TSH plays a critical role in ontogeny (77).

2.4 Thyroid gland and hormone secretion

2.4.1 Definition

The thyroid gland is a bilobed organ of endocrine system located in the neck region. The gland produces thyroid hormones and calcitonin in two distinct cell types, the thyroid follicular cells (TFCS) and the parafollicular or C cells, respectively. The TFCs, the most numerous cell population in the gland, form the thyroid follicles, spherical structures serving as storage and controlled release of thyroid hormones (78).

2.4.2 Thyroid hormones

Thyroid hormones (THs) play critical roles in differentiation, growth, and metabolism. Indeed, TH is required for the normal function of nearly all tissues, with major effects on oxygen consumption and metabolic rate (79). Disorders of the thyroid gland are among the most common endocrine maladies. Furthermore, endemic cretinism due to iodine deficiency remains a public health problem in developing countries. TH synthesis and secretion is exquisitely regulated by a negative-feedback system that involves the hypothalamus, pituitary, and thyroid gland (hypothalamic/pituitary/thyroid (HPT) axis)(80).

THs, T_4 and the more potent T_3, are synthesized in the thyroid gland. Iodide is actively transported and concentrated into the thyroid by Na^+/I^- symporter (NIS)(81,82). The trapped iodide is oxidized by thyroid peroxidase (TPO) in the presence of hydrogen peroxide and incorporated into the tyrosine residues of a glycoprotein, thyroglobulin (Tg). This iodination of specific tyrosines located on Tg yields monoiodinated and diiodinated residues (MIT, monoiodo-tyrosines; DIT, diiodo-tyrosines) that are enzymatically coupled to form T_4 and T_3. The iodinated Tg containing MIT, DIT, T_4, and T_3, then is stored as an extracellular storage polypeptide in the colloid within the lumen of thyroid follicular cells. Genetic defects along the synthetic pathway of THs have been described in humans and are major causes of congenital hypothyroidism in iodine-replete

environments (83,84). The majority of released TH is in the form of T_4, as total serum T_4 is 40-fold higher than serum T_3. Only 0.03% of the total serum T_4 is free (unbound), with the remainder bound to carrier proteins such as thyroxine binding globulin (TBG), albumin, and thyroid binding prealbumin. Approximately 0.3% of the total serum T_3 is free, with the remainder bound to TBG and albumin. It is the free TH that enters target cells and generates a biological response.

The major pathway for the production of T_3 is via 5'-deiodination of the outer ring of T_4 by deiodinases and accounts for the majority of the circulating T_3 (85,86). Type I deioidinase is found in peripheral tissues such as liver and kidney and is responsible for the conversion of the majority of T_4 to T_3 in circulation. Type II deiodinase is found in brain, pituitary, and brown adipose tissue and primarily converts T_4 to T_3 for intracellular use (87). 5'-Deiodination by type I deiodinase and type III deioidinase, which is found primarily in placenta, brain, and skin, leads to the generation of reverse T_3 (rT_3), the key step in the inactivation of TH. rT_3 and T_3 can be further deiodinated in the liver and are sulfo- and glucuronide-conjugated before excretion in the bile (88).

2.4.3 Thyroid hormone receptors

Thyroid receptors (TRs) have been shown to belong to a large superfamily of nuclear hormone receptors that include the steroid hormones (89,90). Although THs may exert their effects on a number of intracellular loci, their primary effect is on the transcriptional regulation of target genes. Early studies showed that the effects of THs at the genomic level are mediated by nuclear TRs, which are intimately associated with chromatin and bind TH with high affinity and specificity (79,91). Similar to steroid hormones that also bind to nuclear receptors, TH enters the cell and proceeds to the nucleus. It then binds to TRs, which may already be prebound to thyroid receptor elements (TREs) located in promoter regions of target genes. The formation of ligand-bound TR complexes that are also bound to TREs is the critical first step in the positive or negative regulation of target genes and the subsequent regulation of protein synthesis (92). TRs share a similar domain organization with other family members as they have a central DNA-binding domain

containing two "zinc fingers" and a carboxy-terminal ligand binding domain (LBD). Initial studies suggested that there were multiple TR isoforms,and subsequent work has confirmed that there are two major TR isoforms encoded on separate genes, designated as TRα and TRβ, encoded on human chromosomes 17 and 3, respectively. Moreover, these multiple isoforms exist in different species such as amphibians, chick, mouse, rat, and human (93). Both TR isoforms bind T_3 and mediate TH-regulated gene expression (94, 95,96).

2.4.4 Nongenomic effects of thyroid hormones

There is general agreement that most of the effects of T_3 are mediated by TR regulation of target gene transcription in the nucleus. However, there are a number of reports on nongenomic effects by T_3 and T_4 (97). Evidence for these nongenomic effects include the lack of dependence on nuclear TRs and structure-function relationships of TH analogs that are different from their affinities for TRs. There also can be rapid onset of action (typically seconds to minutes), and utilization of membrane-signaling pathways, typically involving kinases or calmodulin, that have not been implicated in direct TR function. The putative nongenomic effects by TH are diverse (92).

2.4.5 Thyroid hormone effects on target tissues

TRs are expressed in virtually all tissues, although the relative expression of TR isoforms may vary among tissues (98, 99,100). In addition to this variable expression of TR isoforms in different tissues, the role of TH can vary in different tissues. Indeed, the myriad effects by a single hormone on so many different tissues is surprising and underscores TH's vital role in cellular function (92) . Thus, in addition to its role on the metabolism of macronutrients and overall energy and oxygen consumption,TH also regulates important functions in specific tissues such as:

1- Bone : TH is critical for normal bone growth and development. In children, hypothyroidism can cause short stature and delayed closure of the epiphyses. Biochemical studies have shown that TH can affect the expression of various bone markers in serum, reflecting changes in both bone formation and

resorption (101, 102,103). TH increases alkaline phosphatase and osteocalcin in osteoblasts. TH may act on bone via TH stimulation of growth hormone and insulin-like growth factor I (IGF-I) or by direct effects on target genes (102,104).

2- Heart : TH lowers systemic vascular resistance, increases blood volume, and has inotropic and chronotropic effects on cardiac function (105). The combination of these effects on both the circulation and the heart itself results in increased cardiac output. Hyperthyroid patients have a high output circulation state, whereas hypothyroid patients have low cardiac output, decreased stroke volume, decreased vascular volume, and increased systemic vascular resistance (105). These changes in cardiac function by TH ultimately depend on the regulation of target genes within the heart and indirect effects due to hemodynamic changes by TH. TH enhances overall total protein synthesis in the heart (106,107). Additionally, it regulates the transcription of several specific proteins that are critical for cardiac function such as myosin heavy chain (MHC) genes (106).

3- Liver : TH has multiple effects on liver function including the stimulation of enzymes regulating lipogenesis and lipolysis as well as oxidative processes (79,108). Some of the lipogenic enzymes that are regulated are malic enzyme, glucose-6-phosphate dehydrogenase, and fatty acid synthase (109). It has been appreciated for many years that hypothyroidism is associated with hypercholesterolemia with elevated serum intermediate and low-density lipoprotein (LDL) cholesterol concentrations (110). The major mechanism for these effects may be lower cholesterol clearance resulting from decreased LDL receptors. An additional mechanism may be decreased hepatic lipase activity in hypothyroidism which decreases conversion of intermediate-density lipoproteins to LDL and high-density lipoprotein metabolism (111,112).

4- Pituitary : TH regulates the synthesis and secretion of several pituitary hormones (113).TH also can negatively regulate thyrotropin (TSH) transcription by direct and indirect mechanisms(114). TH can negatively regulate thyrotropin releasing hormone (TRH) at the transcriptional level, which in turn decreases transcription of TSH mRNA (115,116). TH also can

negatively regulate TSH by decreasing transcription of the glycoprotein hormone α-subunit (common to TSH, luteinizing hormone, follicle-stimulating hormone, and human chorionogonadotropic hormone) (114,117 -122).

5- Brain : TH has major effects on the developing brain in utero and during the neonatal period (123,124). Neonatal hypothyroidism due to genetic causes and iodine deficiency in humans can cause mental retardation and neurological defects (125,126).

2.4.6 Thyroid and lipid metabolism

The role of thyroid hormone in the regulation of lipid metabolism has been of interest ever since a relationship between thyroidal state and body weight was identified (127) .The relationship between body fat, thyroidal state, and metabolism became firmly established with the measurement of basal metabolic rates (128). These studies clearly showed that hyperthyroidism is associated with an increase in metabolic rate (oxygen consumption) and that hypothyroidism is associated with a decrease in metabolic rate. Moreover, the changes in metabolic rate were associated with alterations in lipid and carbohydrate metabolism (129). However, the mechanisms leading to these changes are complex because alterations in thyroidal state affect multiple systems and multiple target tissues.

Thyroid hormone regulates the rate of both fat synthesis (lipogenesis) and lipolysis (130). T_3 induces key lipogenic enzymes such as acetyl CoA carboxylase, malic enzyme, glucose-6-phosphate dehydrogenase and fatty acid synthase (131, 132, 133, 134,135). The expression of these genes is also modulated by other factors such as high-carbohydrate diet, insulin, and cAMP (79,136). Researchers demonstrated that the enzymes in the lipogenic pathway are regulated by thyroid hormone in both the liver and adipose tissue (137) Moreover, they showed that thyroid hormone and dietary sucrose act synergistically to control the content of these enzymes (138). With the advent of newer technology, some of the controversial issues discussed above will be answered. In this issue researchers make use of a new microdialysis technique to study the effect of thyroidal state on lipolysis in vivo.

They measured local release of norepinephrine (NE) and showed that (NE) concentrations at the adipocyte are greater in hyperthyroid patients and significantly less in hypothyroid patients compared with euthyroid controls (139).

2.4.7 Increased oxidative energy metabolism

Thyroid hormone has long been recognized as a major regulator of the oxidative metabolism of energy producing substrates by the mitochondria. The mitochondria are often called the "cell's powerhouses" because this is where foodstuffs are turned into useful energy in the form of ATP (140). T3 and diiodotyrosine(T2) increase the flux of nutrients into the mitochondria as well as the rate at which they are oxidized, by increasing the activities of the enzymes involved in the oxidative metabolic pathway. The increased rate of oxidation is reflected by an increase in oxygen consumption by the body.T3 and T2 appear to act by different mechanisms to produce different results. T2 is believed to act on the mitochondria directly, increasing the rate of mitochondrial respiration, with a consequent increase in ATP production. T3 on the other hand acts at the nuclear level, inducing the transcription of genes controlling energy metabolism, primarily the genes for so-called uncoupling proteins, (UCP).

The time course of these two actions is quite different. T2 begins to increase mitochondrial respiration and metabolic rate immediately. T3 on the other hand requires a day or longer to increase BMR since the synthesis of new proteins, the UCP, is required (140). There are a number of putative mechanisms whereby T2 is believed to increase mitochondrial energy production rates, resulting in increased ATP levels. These include an increased influx of Ca++ into the mitochondria, with a resulting increase in mitochondrial dehydrogenases. This in turn would lead to an increase in reduced substrates available for oxidation. An increase in cytochrome oxidase activity has also been observed (141).

2.4.8 Mitochondrial uncoupling

The mitochondria are often characterized as the cell's powerhouse. They convert foodstuffs into ATP, which is used to fuel all the body's metabolic processes. Much research suggests that T3 has the ability to uncouple oxidation of substrates from ATP production. T3 is believed to increase the production of so called uncoupling proteins. Uncoupling protein (UCP) is a transporter family that is present in the mitochondrial inner membrane, and as its name suggests, it uncouples respiration from ATP synthesis by dissipating the transmembrane proton gradient as heat. Instead of useful ATP being produced from energy substrates, heat is generated instead (142).

2.4.9 Adrenergic receptor modulation

Administration of T3 has been shown to upregulate the so - called β 2 adrenergic receptor in fat tissue. What is the significance of this effect for fat loss? Before fat can be used as fuel, it must be mobilized from the fat cells where it is stored. An enzyme called hormone sensitive lipase (HSL) is the rate - controlling enzyme in lipolysis, or fat mobilization. The body produces two catecholamines, epinephrine and nor epinephrine, which bind to the beta 2 receptor and activate HSL. The upregulation of the β 2 receptor due to T3 results in an increased ability of catecholamines to activate HSL, leading to increased lipolysis (143) .

2.4.10 Decreased phosphodiesterase expression

In hyperthyroid patients as well as in normal subjects given T3, levels of the enzyme phosphodiesterase are lowered in fat cells (144). When lipolytic hormones like epinephrine bind to the beta 2 receptor described above, they initiate a signaling cascade mediated by the so called (secend messenger) cyclic AMP (cAMP). cAMP in turn acts on other cellular enzymes to initiate and maintain lipolysis. The original signal is terminated when cAMP is degraded by the enzyme phosphodiesterase. Maintaining elevated cAMP levels, by lowering phosphodiesterase concentrations with T3, will prolong lipolysis (145) .

Chapter 3

Materials and methods

3. Materials and methods

3.1 Study design

The present study is a case – control.

3.2 Study population

The study population comprised two groups, the cases (individuals with BMI >30 kg/m_2), and the controls (non-obese individuals with BMI18.5-24.9 kg/m_2). Cases and controls reside in Gaza Governorate and aged matched (20-40) years.

3.3. Sampling and sample size

Eighty two obese individuals were selected from Gaza governorate and another 82 of normal weight individuals who have the same conditions were selected as a control group. The sample size calculations were based on the formula for case-control studies. EPI-INFO statistical package version 3.5.1 was used with 95% CI, 80% power and 50% proportion as conservative and OR>2. The sample size in case of 1:1 ratio of case control was found to be 73:73. For a no-response expectation, the sample size was increased to 82 obese individuals and the controls also consisted of 82 normal weight individuals.

3.4 Inclusion criteria

A. Case group

1. Men aged 20-40 years old.
2. (BMI) over 30kg/m2.
3. Non diabetic normotensive individuals.

B- Control group

The inclusion criteria for control group are similar to the case group except that they are normal weight.

3.5 Questionnaire

A meeting interview was used for filling in the questionnaire for both cases and controls (Appeddixe1). All interviews were conducted face to face by the researcher himself. During the survey the interviewer explained any of the questions that were not clear. Most questions were yes/no question, which offer a dichotomous choice, and the multiple choice questions. The questions were direct and brief and the validity of the questionnaire was tested by three specialists. The questionnaire included personal information, various obesity related items, thyroid disorders, eating habits and physical activity.

3.6 Pilot study

Pilot study was done prior to beginning real data collection to know the length and clarity of questionnaire and to evaluate the outcome. Ten individuals were interviewed. At the end of the pilot study, a comprehensive revision to questionnaire was made and modified as necessary. The pilot subjects were not included in the study.

3.7 Ethical consideration

An approval to carry out the study was obtained from the Helsinki Committee-Gaza (Appeddixe2). Cases and controls participation was voluntary in the study and all information obtained were kept as confidential.

3.8 Body mass index (BMI)

BMI was used for the evaluation of the obesity. To measure BMI, we weighed the individuals in underclothes and without shoes. Height was measured without shoes. Medical balance (Detecto scale), was used for this purpose. Body mass index is a statistical measurement which compares a person's weight and height, which can be used to indicate if individuals are overweight, obese, underweight or normal.

$$\text{BMI} = \frac{\text{Weight in Kilograms}}{(\text{Height in Meters})^2}$$

BMI Categories:

- Underweight = < (18.5) kg/m2
- Normal weight = (18.5-24.9) kg/m2
- Overweight = (25-29.9) kg/m2
- Obesity = BMI of (30) kg/m2 or greater

3.9 Blood sample collection

Twelve hours fast blood samples were collected from the cases and controls. About 5 ml of venous blood was drawn from each individual. The serum samples were rapidly separated by centrifugation for 10 minute at room temperature at 3500 rpm, the separated samples were frozen at -20 C until assay.

3.10 Biochemical analysis

3.10.1 Determination of insulin

Monobinds insulin MAPS ELISA test kit (Insulin-C peptide/ Product code 7325-300) was used for the quantitative determination of insulin level in human serum.

- Principle

The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (Ab) (enzyme conjugated and immobilized), with different and distinct epitope recognition, in excess, and native antigen (Ag). In this procedure the immobilization takes place during the assay at the surface of the microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal insulin antibody. Upon mixing monoclonal biotinylated antibody, the enzyme labeled antibody and a serum containing the native antigen reaction results between the native antigen and the antibodies without competition or steric hindrance, to form soluble sandwich complex.

Simultaneously the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylateed antibody, after equilibrium is attained the antibody–bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration .

- Reagents

A. Insulin calibrators-2ml/vial(dried),Six (6) vials of references for insulin antigens at levels of 0(A),5(B),25(C),50(D),100(E),and 300(F) (µIU/ml). each vial was reconstituted with 2 ml of distilled water.

B. Insulin enzyme reagent (13ml/vial), One (1) vial containing enzyme labeled affinity purified monoclonal mouse x-insulin IgG, Biotinylatrd monoclonal mouse x-insulin IgG in buffer, dye, and preservative .

C. Streptavidin coated plate (96wells), One 96 well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent.

D. Wash solution concentrate (20 ml), One (1) vial containing a surfactant in phosphate buffered saline, a preservative has been added.

E. Substrate A (7ml/vial), One bottle containing tetramethylbenzidine (TMB) in buffer store at 2-8c

F. Substrate B (7ml/vial), One bottle containing hydrogen peroxide (H_2O_2) in buffer.

G. Stop solution (8ml /vial), One bottle containing a strong acid (1N HCL).

- Reagent preparation

1. Wash buffer, contents of wash solution were diluted to 1000ml with distilled water in a suitable storage container.

2. Working substrate solution, the contents of the amber vial labeled solution A were added into the clear vial labeled solution B. mixed and labeled accordingly.

- Assay procedure

Before proceeding with the assay all reagents, serum, references and controls were brought to room temperature.

1. Microplates wells for calibrator, control and patient specimen were assayed in duplicate.

2. 50 µl of the appropriate calibrators, controls and samples were pipeted into the assigned wells.

3. 100 µl of the insulin enzyme reagent were added to each well.

4. The microplate was swirled gently for 20-30 seconds in order to mix and covered with plastic wrap.

5. The microplate was Incubated for 120 minutes at room temperature.

6. The contents of the microplate were discarded by decantation or aspiration.

7. 300 µl of wash buffer were added, decant or aspirate. An automatic washer was used.

8. 100 µl of working substrate solution were added to all wells.

9. The microplate was Incubated at room temperature for 15 minutes.

10. 50 µl of stop solution were added to all wells and mixed gently for 15-20 seconds.

11. The absorbance was measured at 450 nm in a microplate ELISA reader.

- Insulin normal range

children < 12	< 10 µIU/ml
adult normal	0.7-9 µIU/ml
Diabetic (type2)	0.7-25 µIU/ml

3.10.2 Determination of thyroid stimulating hormone (TSH)

The Teco Thyroid Stimulating Hormone ELISA test kit was used for the quantitative determination of thyroid stimulating hormone (TSH) concentration in serum.

- Principle

The essential reagents required for an immunoenzymometric assay include excess amount of antibodies (both enzyme conjugated and immobilized) with high affinity, high specificity and contain different epitopes with distinct recognition and native antigen. In this assay procedure, the immobilization takes place at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-TSH antibody. Upon mixing, a reaction results between the native antigen contained in serum, the monoclonal biotinylated antibody and the enzyme-labeled antibody, without competition or steric hindrance, to form a soluble sandwich complex. Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration.

- Reagents

1. Streptavidin coated microplate: containing 96 wells per kit

2. Enzyme Conjugate: containing TSH antibody conjugated with HRP and biotinylated antibody in buffer, 13 ml.

3. TSH Reference Standard set*:* (7 1-mL vials) containing 0, 0.5, 2.5, 5.0, 10, 20 and 40 µIU/ml TSH antigen in buffer. Exact concentrations are given on the package labels on a lot specific basis.

4. TMB Solution: containing TMB and H_2O_2 reagent in amber bottle, 11 ml.

5. Stop Solution: containing diluted hydrochloric acid, 8 ml.

6. Wash Solution concentrate: 20 ml.

Reagent preparation

1. All reagents were allowed to reach room temperature before use.

2. Working wash solution: 20 mL of wash solution concentrate were added to 1000 mL of deionized water, and mixed well.

- Assay procedure

1. The desired number of coated wells were secured in the holder.

2. 50 µl of standards, sample and controls were pipetted into each well.

3. 100 µl of conjugate reagent were pipetted into each well, and mixed thoroughly for 30 seconds.

4. The microplate was incubated at room temperature for 60 minutes.

5. The contents of the wells were discarded by decantation or aspiration.

6. 300 µl of working wash solution were pipetted. An automatic ELISA washer was used.

7. One hundred µl of TMB reagent were added into each well, and gently mixed for 10 seconds.

9. The microplate was Incubated at room temperature in the dark for 15 minutes without shaking.

10. 50 µl of stop solution were pipetted to each well and gently mixed for 10-20 seconds. It is critical to make sure that the blue color was changed to yellow color completely.

11. The absorbance in each well was measured at 450 nm using ELISA reader.

- **Expected values**

Expected values	0.4 – 6.0 µIU/ml

3.10.3 Determination of Glucose

Serum glucose was determined by enzymatic colorimetric method for the quantitative determination of glucose in blood using Globe diagnostics kit, (Italy).

Principle

Glucose is transformed by glucose oxidase (GOD) into gluconic acid and hydrogen peroxide, which in presence of peroxidase (POD), reacts with phenol and 4-aminoantipirine to form a red complex, whose intensity at 510 nm is proportional to the glucose concentration in the sample.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{Phenol} + \text{4-Aminoantipirine} \xrightarrow{\text{POD}} \text{Red complex} + H_2O$$

- Reagents

- Reagent A

Phosphate buffer pH (7.4)	25 g/l
Phenol	< 0.9 g/l
4-Aminoantipirine	0.4 mmol/l
GOD	≥ 30 kU/l
POD	≥ 1 kU/l
NaN_3	0.95 g/l

-Standard

D-Glucose	100 mg/dl (5.55mmol/l)
Benzoic acid	< 14.7 mmol/l

Assay procedure

About 0.5 ml of serum was transferred to the Mindray BS-120 chemistry autoanalyzer to perform the test according to these parameters **:**

Parameter	Value
Reagent (µl)	300
Serum (µl)	3
Incubation period (s)	17×18
Reaction type	End point
Wavelength (nm)	510

- Reference values

Adults	70-115 mg/dl
Newborn	20-80 mg/dl
Children<5 Years	values 10-15% lower of adults ones

3.10.4 Determination of total cholesterol

Total Cholesterol was determined by enzymatic colorimetric method for the quantitative determination of total cholesterol in serum or plasma, using Globe diagnostics kit, (Italy).

Principle

The measurement is based on the following enzymatic reactions**:**

$$\text{Cholesterol esters} + H_2O \xrightarrow{CHE} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{CHOD} \text{Cholest-4-en-3-one} + H_2O_2$$

$$2\ H_2O_2 + \text{hydroxybenzoate} + \text{4-Amminoantipyrine} \xrightarrow{POD} \text{Red complex} + 4H_20$$

The intensity of the red complex is proportional to the total cholesterol present in the sample.

- Reagents

- Reagent A

Good buffer, pH (6.7)	50 mmol/l
Cholesterol oxidase (CHOD)	≥ 100 U/l
Cholesterol esterase (CHE)	≥ 300 U/l
Hydroxybenzoic acid	12 mmol/l
4-Amminoantipirine	0.3 mmol/l
Peroxidase (POD)	≥ 500 U/l
Sodio azide	≤ 0.095 g/l

- Standard

Cholesterol	200 mg/dl

Assay procedure

About 0.5 ml of serum was transferred to the Mindray BS-120 chemistry autoanalyzer to perform the test according to these parameters :

Parameter	Value
Reagent (µl)	300
Serum (µl)	3
Incubation period (s)	17×18
Reaction type	End point
Wavelength (nm)	510

Reference range

Cholesterol values according to a study on a population of adults in absence of coronary disease are the following :

Recommended values	< 200 mg/dl
Upper limit	200 – 239 mg/dl
High value	≥ 240 mg/dl

3.10.5 Determination of triacylglycerol

Triacylglycerol were determined by enzymatic colorimetric method, for the quantitative determination of Triacylglycerol in serum, plasma using Globe diagnostics kit ,(Italy).

Principle

Glycerol, released from triglycerides after hydrolysis with lipoproteinlipase is ransformed by glycerolkinase into glycerol-3-phosphate which is oxidized by glycerolphosphate oxidase into dihydroxyacetone phosphate and hydrogen peroxide. In presence of peroxidase, the hydrogen peroxide oxidizes the chromogenESPT (4-aminophenazone/N-ethylmethylanilin- propan-sulphonate sodic) to form purple quinoneimine whose colour intensity, measured at 510 nm, is proportional to the concentration of triglycerides in the sample.

$$\text{Triacylglycerol} \xrightarrow{\text{LPL}} \text{Glycerol + Fatty acids}$$

$$\text{Glycerol + ATP} \xrightarrow{\text{GK}} \text{Glycerol-3-phosphate + ADP}$$

$$\text{Glycerol-3-phosphate + } O_2 \xrightarrow{\text{GPO}} \text{Dihydroxyacetone phosphate + } H_2O_2$$

$$2\ H_2O_2 + \text{Amminoantipirine + ESPT} \xrightarrow{\text{POD}} \text{Quinoneimine + HCl + } 4\ H_2O$$

- Reagents

- Reagent A

Good Buffer pH (7.2)	50 mmol/l
ESPT	4 mmol/l
ATP	2 mmol/l
Mg++	2 mmol/l
Lipoproteinlipase (LPL)	>1 kU/l
Glycerol kinase (GK)	> 0.4 kU/l
Glycerolphosphate oxidase (GPO)	>1.5 kU/l
4-Amminoantipirine	0.5 mmol/l
Peroxidase (POD)	>1 kU/l
NaN3	< 0.095 g/l

- Standard

Glycerol	200 mg/dl
NaN3	≤ 0.095g/l

Assay procedure

About 0.5 ml of serum was transferred to the Mindray BS-120 chemistry autoanalyzer to perform the test according to these parameters :

Parameter	Value
Reagent (µl)	300
Serum (µl)	3
Incubation period (s)	17×18
Reaction type	End point
Wavelength (nm)	510

Reference range

Recommended values	< 200 mg/dL
Upper limit	200-400 mg/dl
High values	> 400 mg/dl

3.10.6 Determination of high density lipoprotein cholesterol (HDL-c)

HDL cholesterol was determined by liquid HDL precipitant for the determination of HDL cholesterol using Globe diagnostics kit, Italy.

- Reagents

- Precipitating Reagent

PEG 6000	14.5%
Surfactants and preservative	

- Assay procedure

1- 0.5 ml serum and 0.5 ml precipitating reagent were pipetted into conic test tubes.

2. Then mixed gently by inversion, and wait 5 minutes and centrifuged at (3000 rpm) for 20 minutes.

3. The supernatant was removed for the HDL cholesterol determination as follows:

- About 0.5 ml of supernatant was transferred to the Mindray BS-120 chemistry autoanalyzer to perform the test according to these parameters:

Parameter	Value
Reagent (µl)	200
supernatant (µl)	3
Incubation period (s)	17×18
Reaction type	End point
Wavelength (nm)	510

- Reference value

Based on the risk for heart diseases the sequent reference ranges are suggested :

Low value (high risk)	Medium value (moderate risk)	High value (low risk)
< 40 mg/dl	40 – 59 mg/dl	> 60 mg/dl

3.10.7 Determination of Low density lipoprotein – cholesterol (LDL- c)

LDL-c was calculated from the primary measurements using the empirical equation

LDL-c (mg/dl) = total cholesterol – triglyceride/5 – HDL-c

3.11 Statistical analysis

Data obtained was arranged and entered to computer to be statistically analyzed using Statistical Package for the Social Sciences (SPSS) program (version 18) . Data analysis was carried out as follows :

1. Data cleaning
2. Frequency table for all the study variables.
3. Defining and recording of certain variables.
4. Cross tabulation and advanced statistical analysis.

The following tests were applied

1. ANOVA test
2. Chi-square test
3. T- test
4. Spearman correlation coefficient
5. Graphs were plotted
6. Percentage difference was calculated according to the formula :

$$\frac{\text{Mean of cases – mean of controls}}{\text{mean of controls}} \times 100$$

Chapter 4

Results

4. Results

4.1 Questionnaire data

4.1.1 General description of the study population

The study population comprised 82 obese individuals (case group), and 82 control individuals. Table 4.1 shows the general characteristics of the study population. The age of the study population ranged from 20-40 years with mean of (27.1±5.2) years for controls and (27.4 ±5.6) years for cases. Fifty one (62.1%) and 29 (35.4%) of controls and cases were single whereas 31(37.9%) and 53 (64.6%) were married. Analysis of the educational status showed that 3 (3.6%) controls and 4 (4.9%) cases were illiterate, 10 (12.4%) and 14 (17.0%) had passed primary school,19 (23.1%) and 17 (21.0%) had finished preparatory school, 23 (28.0%) and 19 (23.1%) had finished secondary school and 27 (32.9%) and 28 (34.0%) had a university degree. A total of 47 (57.3%) controls and 51 (62.2) cases were employed whereas 35 (42.7%) and 31 (37.8%) were unemployed. In addition, 22 (26.8%) controls and 20 (24.4%) cases were smokers.

Table 4.1. General characteristics of the study population

Character	Controls (n=82)		Cases (n=82)	
	No.	%	No.	%
Age (Year)				
20-26	45	54.9	42	51.2
27-33	25	30.5	27	32.9
34-40	12	14.6	13	15.9
mean±SD	(27.1±5.2) Years		(27.4 ±5.6) Years	
Marital status				
Single	51	62.1	29	35.4
Married	31	37.9	53	64.6
Education				
Illiterate	3	3.6	4	4.9
Primary school	10	12.4	14	17.0
Preparatory school	19	23.1	17	21.0
Secondary school	23	28.0	19	23.1
University	27	32.9	28	34.0
Employment				
Employed	47	57.3	51	62.2
Unemployed	35	42.7	31	37.8
Smoking				
Yes	22	26.8	20	24.4
No	60	73.2	62	75.6

4.1.2 Various obesity related items as reported by the study population

Table 4.2 provides various obesity related items as reported by the study population. A total number of 14 (17.1%) cases had history of hyperlipidemia compared to only one control (1.2%). The difference was significant with χ^2 =10.566 and p=0.001. The number of cases who reported suddenly weight gain 38 (46.3%) or trying to lose weight but failed 19 (23.2%) were significantly higher than controls 2 (2.4%) and 0 (0.0%), respectively (χ^2 =40.504, p=0.000 and χ^2 =19.287, p=0.000). Seventy (85.4%) cases indicated obesity among their first degree relatives in comparison to 30 (36.6%) controls who did (χ^2 =41.0, p=0.000) indicating that family history is a risk factor for obesity.

Table 4.2. Various obesity related items as reported by the study population

Obesity Items	Controls (n=82)		Cases (n=82)		χ^2	P-value
	No.	%	No.	%		
History of hyperlipidemia						
Yes	1	1.2	14	17.1	10.566	0.001*
No	81	98.8	68	82.9		
Suddenly weight gain						
Yes	2	2.4	38	46.3	40.504	0.000*
No	80	97.6	44	53.7		
Trying to lose weight but failed						
Yes	0	0.0	19	23.2	19.287	0.000*
No	82	100	63	76.8		
Obesity among first degree relatives						
Yes	30	36.6	70	85.4	41	0.000
No	52	63.4	12	14.6		

* p value of $\chi^2_{(corrected)}$ test

4.1.3 Thyroid disorders

Table 4.3 summarizes thyroid disorders among the study population. The controls and cases who reported getting fatigue quickly were 8 (9.8%) and 37 (45.1%) respectively compared to 74 (90.2%) and 45 (54.9%) who did not get fatigue (χ^2 =25.756, p=0.000). Muscle weakness was higher in cases; 15

(18.3%) than in controls; 4 (4.9%) with χ^2 =5.953 and p=0.015. In addition, consumption of suitable quantities of marine food was lower in cases; 19 (23.2%) compared to controls; 34 (41.5) with χ^2 =6.272 and p=0.012. This indicates that previous factors are more likely to be associated with obesity. The remaining disorders including decreased sweating, slow heart rate, difficulty in concentrating or remembering things, sleep problems and feeling lazy when waking up showed no significant differences between cases and controls (p>0.05).

Table 4.3. Thyroid disorders among the study population

Item	Controls (n=82)		Cases (n=82)		χ^2	P-value
	No.	%	No.	%		
Treatment of thyroid gland problems						
Yes	0	0	1	1.2	NA*	NA*
No	82	100	81	98.8		
Getting fatigue quickly						
Yes	8	9.8	37	45.1	25.756	0.000
No	74	90.2	45	54.9		
Decreased sweating						
Yes	6	7.3	11	13.4	1.641	0.200
No	76	92.7	71	86.6		
Suffer from muscle weakness						
Yes	4	4.9	15	18.3	5.953	0.015**
No	78	95.1	67	81.7		
Slow heart rate						
Yes	1	1.2	3	3.7	0.256	0.613**
No	81	98.8	79	96.3		
Consumption of suitable quantities of fishes						
Yes	34	41.5	19	23.2	6.272	0.012
No	48	58.5	63	76.8		
Difficulty in concentrating or remembering things						
Yes	21	25.6	32	39.0	3.373	0.066
No	61	74.4	50	61.0		
Sleep problems						
Yes	15	18.3	19	23.2	0.594	0.441
No	67	81.7	63	76.8		
Feeling lazy when waking up						
Yes	36	43.9	38	46.3	0.098	0.754
No	46	56.1	44	53.7		

* Non applicable

** p value of χ^2 (corrected) test

4.1.4 Eating habits among the study population

Table 4.4 illustrates eating habits among the study population. A total number of 44 (53.7%) cases mentioned that they eat large quantities of carbohydrate and lipid in comparison to their counterparts of 28 (34.1%). The difference between the two groups was significant (χ^2 =6.338 and p=0.012) implying that eating large quantities of carbohydrate and lipid are associated with obesity. However, no significant differences were observed between cases and controls for eating just before bed time and snacking between meals (χ^2 =3.310, p=0.069 and χ^2 =0.098, p=0.754, respectively).

Table 4.4. Eating habits among the study population

Item	Controls (n=82)		Cases (n=82)		χ^2	P-value
	No.	%	No.	%		
Eating large quantities of carbohydrate and lipid						
Yes	28	34.1	44	53.7	6.338	0.012
No	54	65.9	38	46.3		
Eating just before bed time						
Yes	49	59.8	60	73.2	3.310	0.069
No	33	40.2	22	26.8		
Snacking between meals						
Yes	43	52.4	41	50.0	0.098	0.754
No	39	47.6	41	50.0		

4.1.5 Physical activity among the study population

The Physical activity among the study population is illustrated in Table 4.5. The number of cases who had regular exercise 9 (11.0%), walking regularly 18 (22.0%) and having active job 22 (26.8%), were less than their counterparts of controls 15 (18.3%), 45 (54.9%) and 36 (43.9%), respectively. However, the difference was significant for walking regularly and having active job (χ^2 =18.789, p=0.000 and χ^2 =5.228, p=0.022, respectively).

Table 4.5. Physical activity among the study population.

Physical activity	Controls (n=82)		Cases (n=82)		χ^2	P-value
	No.	%	No.	%		
Regular exercise						
Yes	15	18.3	9	11.0	1.757	0.185
No	67	81.7	73	89.0		
Walking regularly						
Yes	45	54.9	18	22.0	18.789	0.000
No	37	45.1	64	78.0		
having active job						
Yes	36	43.9	22	26.8	5.228	0.022
No	46	56.1	60	73.2		

4.2 Biochemical parameters of the study population

4.2.1 Insulin, glucose and thyroid stimulating hormone

Table 4.6 shows the levels of Insulin, glucose and thyroid stimulating hormone (TSH) in controls and cases. The mean levels of insulin and glucose were significantly increased among cases compared to controls (mean=13.0±13.0 and 91.0±20.2 Vs 6.9±8.0 and 83.7±11.2, % difference=88.4 and 8.7, P=0.000 and 0.005, respectively). In contrast, the mean levels of TSH were lower in cases compared to controls (mean=1.41±1.23 Vs 1.93±0.98, % difference=26.9 and P=0.003.

Table 4.6. Insulin, glucose and thyroid stimulating hormone of the Study population

Parameter	Control (n=82) mean±SD	Case (n=82) mean±SD	% difference	t	P-value
Insulin (µIU/mL) *(min-max)*	6.9±8.0 *(0.4-40.3)*	13.0±13.0 *(0.7-90)*	88.4	3.631	0.000
Glucose (mg/dl) *(min-max)*	83.7±11.2 *(67-130)*	91.0 ±20.2 *(61-190)*	8.7	2.874	0.005
TSH* (µIU/mL) *(min-max)*	1.93±0.98 *(0.3-6.1)*	1.41± 1.23 *(0.1-7.1)*	-26.9	-2.983	0.003

TSH*: Thyroid stimulating hormone.
All values are expressed as mean ±SD.
P<0.05: significant.

4.2.2 lipid profile of the study population

Lipid profile including, total cholesterol, triacylglycerol, high density lipoproteins (HDL) and low density lipoproteins (LDL) are listed in Table 4.7. The mean levels of total cholesterol, triacylglycerol and LDL increased in cases compared to controls (mean=187.3±38.5mg/dl,139.2±63.7mg/dl and 119.4±34.8mg/dl Vs 174.9±43.5mg/dl, 98.9±68.6mg/dl and 107.9±42.4mg/dl, % difference=7.1, 40.7 and 10.6, respectively). However, the difference was significant only for triacylglycerol (P=0.000). In contrast, the mean level of HDL was significantly decreased in cases compared to controls (40.1±10.9mg/dl Vs 47.7±9.6mg/dl, % difference=15.9 and P=0.000)

Table 4.7. lipid profile of the study population

Lipid profile (mg/dl)	Control (n=82) mean±SD	Case (n=82) mean±SD	% difference	t	P-value
Total cholesterol *(min-max)*	174.9±43.5 *(89-328)*	187.3±38.5 *(87-264)*	7.1	1.940	0.054
Triacylglycerol *(min-max)*	98.9±68.6 *(37-610)*	139.2±63.7 *(35-323)*	40.7	3.900	0.000
HDL* *(min-max)*	47.7±9.6 *(27-79)*	40.1±10.9 *(22-79)*	-15.9	-4.723	0.000
LDL** *(min-max)*	107.9±42.4 *(21-273)*	119.4±34.8 *(27-201)*	10.6	1.911	0.058

HDL*: High density lipoprotein, LDL**: Low density lipoprotein.
All values are expressed as mean ±SD.
p<0.05 :significant, P>0.05 not significant.

4.3 Distribution of different grades of body mass index (BMI) among cases

Table 4.8 illustrates the distribution of different grades of body mass index (BMI) among cases (obese individuals). Twenty seven (32.9%) of cases were found to be obese grade 1, 27 (32.9%) were obese grade 2 and 28 (34.2%) were obese grade 3.

Table 4.8. Distribution of different grades of body mass index (BMI) among cases

Body mass index (Kg/m^2)	No.	%
Grade 1 (30-34.9)	27	32.9
Grade 2 (35-39.9)	27	32.9
Grade 3 (≥ 40)	28	34.2

Weight classifications by BMI: people with BMI=(30-34.9) were considered obese grade I and those with BMI=(35-39.9) were obese grade II, and people with BMI=≥40 were classified as morbid obese of grade III (47).

4.4 Body mass index in relation to different biochemical parameters of cases

Table 4.9 presents the relation between (BMI) and the studied biochemical parameters including insulin, TSH, glucose, total cholesterol, triacylglycerol, HDL and LDL of cases. There was no significant relation between these parameters and the different grades of obesity (P>0.05)

Table 4.9. Categories of body mass index in relation to different biochemical parameters of cases.

Parameter	Obesity grade (BMI)			F	P-value
	Grade 1 (n=27) mean±SD	Grade 2 (n=27) mean±SD	Grade 3 (n=28) mean±SD		
Insulin (µIU/mL)	15.2±21	11.9±7.6	12.1±4.3	0.546	0.432
TSH* (µIU/mL)	1.4±1.4	1.4±1	1.5±1.5	0.054	0.947
Glucose (mg/dl)	90.7±25.6	95.2±20.3	87.4±13.1	1.027	0.363
Total cholesterol (mg/dl)	192.5±41.8	180.9±33.1	188.7±40.6	0.636	0.532
Triacylglycerol (mg/dl)	147.3±67.4	123.2±55.5	146.9±66.8	1.279	0.284
HDL (mg/dl)**	38.7±10.3	40.8±11.1	40.9±11.6	0.358	0.700
LDL* (mg/dl)**	124.3±11.6	115.6±31.1	118.6±37	0.432	0.651

TSH*: Thyroid stimulating hormone, HDL**: High density lipoprotein, LDL***:Low density lipoprotein

4.5 Insulin Categories in relation to different biochemical parameters of cases

The relations of Insulin to the studied biochemical parameters of cases are illustrated in Table 4.10. Insulin level was classified in three categories: <9, 9-18 and >18 µIU/mL. A total number of 50 (61%) patients were hyperinsulinemic (insulin level ≥9 µIU/mL). The increases in the mean levels of insulin throughout the three categories of <9, 9-18 and >18 were significantly associated with increasing glucose levels (F=5.619, P=0.005). On the other hand, the relation of insulin with other parameters was not significant (P>0.05).

Table 4.10. Insulin Categories in relation to different biochemical parameters of cases

Parameter	Insulin level (µIU/mL)			F	P-value
	(<9) mean±SD	(9-18) mean±SD	(>18) mean±SD		
Glucose (mg/dl)	88.2±18.7	89.1±18.7	112.5±34.3	5.619	0.005
TSH* (µIU/mL)	1.2±1.17511	1.5±1.3	1.2 ±1.4	0.184	0.832
Total cholesterol (mg/dl)	188.1±37.8	187±38.9	185.8 ±44.6	0.015	0.985
Triacylglycerol (mg/dl)	135.5±65.6	142.5±64.1	138.4±61	0.110	0.896
HDL (mg/dl)**	40.7±11.7	40.7 ±10.9	34.9±6.8	1.018	0.366
LDL* (mg/dl)**	120.5±33	117.8 ±37.5	123.4±32.3	0.108	0.898

TSH*: Thyroid stimulating hormone, HDL**: High density lipoprotein, LDL***: Low density lipoprotein

4.6 Categories of thyroid stimulating hormone in relation to different biochemical parameters of cases

Table 4.11 represents the relation between TSH and the studied biochemical parameters including, glucose, total cholesterol, triglycerides, HDL and LDL of cases. There was no significant relation between these parameters and the different TSH levels (P>0.05).

Table 4.11. Categories of TSH versus glucose and lipid profile

Parameter	TSH level (µIU/mL)			F	P-value
	(<0.5) mean±SD	0.5-1.5) mean±SD	(>1.5) mean±SD		
Glucose (mg/dl)	89±27.9	89±14.5	92.2±22.3	0.129	0.879
Total cholesterol (mg/dl)	192.2±35.2	188.8±35.4	182.3±45.1	0.384	0.682
Triacylglycerol (mg/dl)	143.4±71	144.3±60.4	129.4±64.9	0.473	0.625
HDL (mg/dl)**	37.3±9	41±10.9	40.7±12.1	0.708	0.496
LDL* (mg/dl)**	126.5±29	118.9±35.1	115.9±38.3	0.484	0.618

HDL**: High density lipoprotein, LDL***: Low density lipoprotein

4.7 Correlation between insulin levels with glucose and lipid profile of cases

Table 4.12 shows the correlation between insulin levels with glucose and lipid profile of cases. Insulin level showed strong positive correlation with glucose level (r=0.470). This correlation was significant with P=0.000 (Figure 4.1). However, a very weak correlations were found with other parameters.

Table 4.12. The correlation of insulin with glucose and lipid profile

parameter	R	p-value
Glucose (mg/dl)	0.470	0.000
Total cholesterol (mg/dl)	-0.023	0.840
Triacylglycerol (mg/dl)	0.029	0.797
HDL (mg/dl)**	-0.121	0.280
LDL* (mg/dl)**	0.003	0.980

HDL**: High density lipoprotein, LDL***: Low density lipoprotein

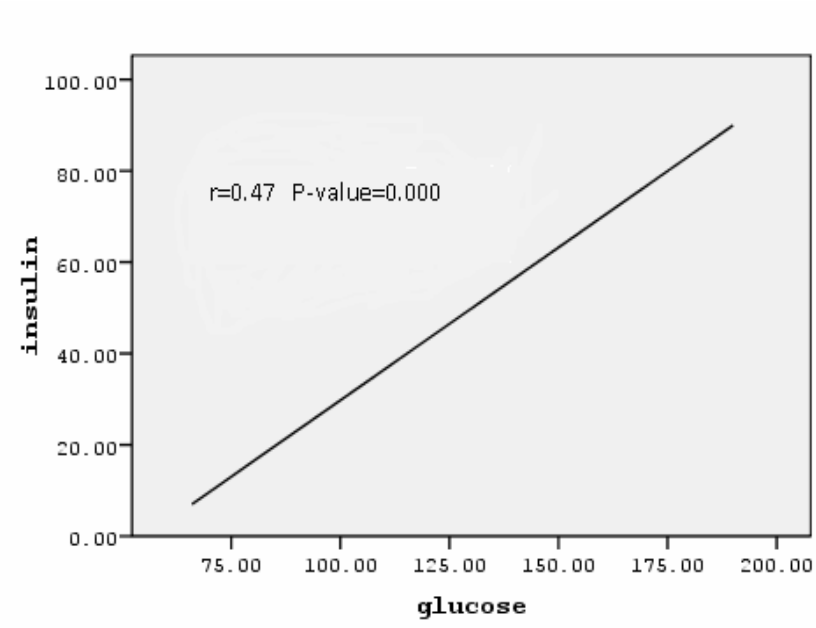

Figure 4.1. Correlation of insulin with glucose

4.8 Correlation between TSH levels with glucose and lipid profile of cases

Table 4.13 shows the correlation between TSH levels with glucose and lipid profile of cases. TSH level showed relatively week negative correlations with total cholesterol and LDL levels (r=-0.233, r=-0.227, respectively). Such correlations were significant with P=0.036 and P=0.041, respectively (Figure 4.2 and Figure 4.3). However, a very weak correlations were found with other parameters.

Table 4.13. The correlation of thyroid stimulating hormone with glucose and lipid profile.

parameter	R	p-value
Glucose (mg/dl)	-0.037	0.742
Total cholesterol (mg/dl)	-0.233	0.036
Triacylglycerol (mg/dl)	-0.048	0.668
HDL (mg/dl)**	-0.037	0.743
LDL* (mg/dl)**	-0.227	0.041

HDL**: High density lipoprotein, LDL***: Low density lipoprotein

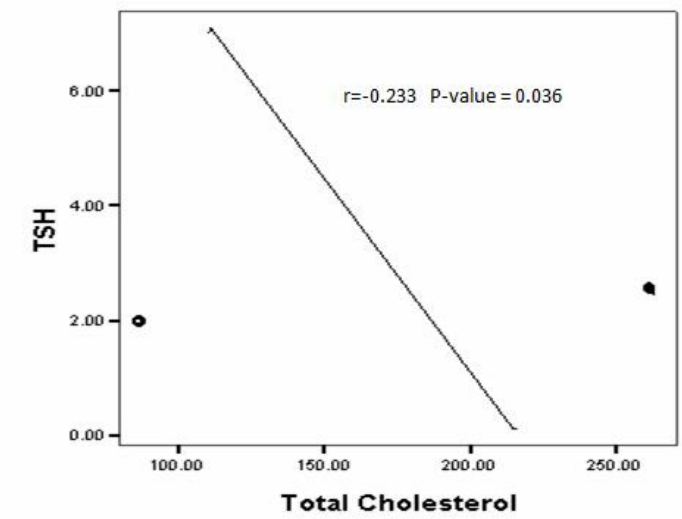

Figure 4.2. Correlation of thyroid stimulating hormone (TSH) with total cholesterol.

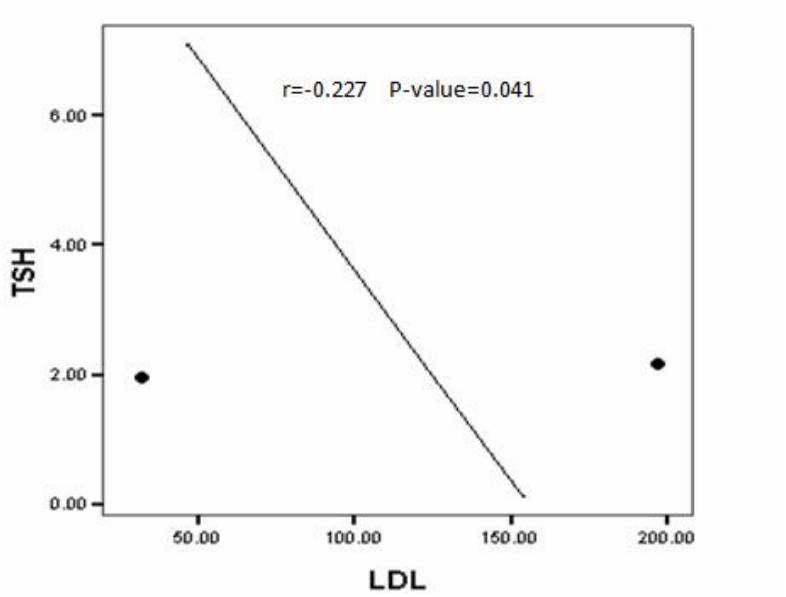

Figure 4.3. Correlation of thyroid stimulating hormone (TSH) with low density lipoprotein (LDL).

Chapter 5

Discussion

5. Discussion

The present study is a case control investigation included 82 obese individuals (BMI >30 kg/m2) and 82 control (non obese individuals, BMI=18.5-24.9 kg/m2) matched with age. The main objective was to assess insulin, thyroid stimulating hormone, glucose and lipid levels among obese adult males in Gaza Governorate. To our knowledge this is the first study in Gaza strip that investigate the relation between obesity, insulin, TSH and lipid profile. The results will benefit obese individuals to be aware of the causes and risk factors related to their obesity and their dangerous consequences.

The age of the study population was ranged from 20-40 years with a mean value of 27.1±5.2 for controls and 27.4 ±5.6 for cases. The results showed that a low level of illiteracy was recorded among controls and cases which reflected a well educated community. More than one third of the study population were found to be unemployed reflecting the unemployment crisis in Gaza Strip. Giacaman et al., (2009) reported that the unemployment in Gaza Strip was around 33% of the active workforce in 2007, and rose to 37% in 2008. Such unemployment crisis is expected to exceed 50% in 2011 as a result of the continuous siege on economic conditions in Gaza Strip. In both controls and cases smokers represented 22 (26.8%) controls and 20 (24.4%) cases. This excluded smoking as a confounder factor of obesity.

The present study showed that the number of cases having a history of hyperlipidemia, reporting suddenly weight gain and trying to lose weight but failed were significantly higher than controls. This result indicated that the above parameters associated with obesity. In addition, the present data showed that most cases have more obese first degree relatives compared to controls implying that obesity is related to genetic factors in this group. The relative contributions of genetics and environment to the etiology of obesity have been evaluated before (35,36). Although such contribution varies from study to another, 30% to 40% of the variance in BMI can be attributed to genetics and 60% to 70% to environment. In a given population, some people

are genetically predisposed to develop obesity, but that genotype may be expressed only under certain adverse environmental conditions, such as high-fat diets and sedentary lifestyles (146) . Increasing numbers of people are being exposed to these adverse environmental conditions, and consequently, the percentage of people expressing the obesity genotype has increased. Such results was supported by the study findings that the number of cases eating large quantities of carbohydrate and lipid was significantly higher than controls .In addition, lower number of cases were found to have insedentary lifestyles.

As revealed by questionnaire, higher percentage of cases were getting fatigue quickly compared to controls. Getting fatigue quickly among obese individuals may be due to hormonal and enzymatic disturbances which affect many biochemical processes in the body. Also lipid accumulation in liver and muscles may play a critical role in the physiology of these organs, and may causes fatigue. In addition, lower number of cases consumed suitable amount of marine food, which is a healthy diet and may promote the synthesis of thyroid hormones (147,148,149,150).

Concerning eating habits among the study population, the results showed that higher percentages of cases ate large quantities of carbohydrate , lipid and just before bed time compared to their counterparts. It is common in our society that most people like to eat traditional sweets (Kunafa) after meals. Such high calories food is a risk factor for obesity. It is well documented that consumption of carbohydrates and lipid-rich diet contributes largely to obesity (41). Eating just before bed time may be another risk factor for obesity. This results is in agreement with other studies (151,152) who found that eating just before bed time contributes to weight gain. They explained that by the idea that the basal metabolic rate (BMR) is low at bed time as the metabolic processes and energy expenditure.

The physical activity among the study population showed that the number of cases who are walking regularly and having active job was significantly less than controls. This indicated that practicing such activities help in weight

reduction i.e. decrease obesity incidence. Such result was consistent with a previous study which proved that sedintary lifestyle and inactive job causes obesity. The etiology of obesity was explained on the basis of low metabolism and low oxygen consumption (40).

Results presented here also indicated that the mean levels of insulin and glucose were significantly higher in cases than in controls. These findings were in agreement with other studies (153). It is worth mentioning that 50 cases were hyperinsulinimic and 6 cases were hyperglycemic. All hyperglycemic cases were hyperinsulinimic but not vice versa. Only one individual in the control group was hyperglycemic and hyperinsulinimic. Hyperinsulinemia may be due to development of insulin resistance (6,7,60,61). However, genetic or other deep studies to investigate the presence of mutations or other changes in the insulin receptors shape are needed. Most cases with insulin resistance are able to secrete enough insulin to maintain non-diabetic glucose levels. Some cases will go on to develop overt type 2 diabetes (154). It is known that obesity is a risk factor for diabetes (6). However, the majority, even if they do not develop diabetes, are still at significantly increased risk for heart attack, stroke and other diseases (155,156). Concerning TSH, results showed lower levels of TSH in cases than in controls. From the general view of the results there was only one patient who had TSH value higher than normal range, and also another one in control group .

The present results showed that the mean levels of total cholesterol, triacylglycerol and LDL were increased in cases compared to controls. Such values reached significant value for triglycerides. These results were in consistent with the previous studies (12,13,14,15). The higher concentrations of serum lipids in cases is mainly due to the observed insulin resistance developed in obese individuals. Several mechanisms whereby insulin resistance could cause an alteration in lipid metabolism have been described. Hyperinsulinemia is known to enhance hepatic very-low-density lipoprotein synthesis and thus may directly contribute to the increased plasma triacylglycerol and LDL cholesterol levels (157). Resistance to the action of

insulin on lipoprotein lipase in peripheral tissues may also contribute to the elevated triacylglycerol and LDL cholesterol levels (158,159) . In contrast, the mean level of HDL was significantly higher in controls than in cases. Lipid abnormalities had been reported in obese adults, who had elevated triacylglycerol and LDL cholesterol and low levels of HDL cholesterol (160,161) . In addition, insulin resistance observed in the present study might also be implicated in the association between obesity and dyslipidemia (162). The lower physical activity reported by cases may further contributes to lower level of HDL and higher level of LDL observed in the present study.

BMI was a major criterion in the selection of study population: case group was selected to be over 30 kg/m2 and control group between 18.5-24.9 kg/m2 (47). In order to make precise relations with biochemical parameters, BMI of individuals in case group was classified into three categories: grade1, grade 2 and grade 3. There was no statistical significant correlations between the studied biochemical parameters including insulin, glucose, TSH, total cholesterol, triacylglycerol, LDL and HDL among different grades of obesity. This reflects a narrow range of change of such parameters among different grades of obesity. This point needs further investigation.

To investigate the relation of Insulin to the studied biochemical parameters glucose, TSH, total cholesterol, triglyceride, LDL and HDL of cases, insulin level was classified into three categories: <9, 9-18 and >18 µIU/mL. The observed increases in the mean levels of insulin throughout the three categories were significantly associated with increasing glucose levels whereas the relation of insulin with other parameters was not significant. This result was supported by strong positive correlation between insulin and glucose levels and a weak correlations with other parameters. Simultaneous increase in insulin and glucose levels in obese individuals might result from development of insulin resistance (6,7,60,61). Most cases with insulin resistance are able to secrete enough insulin to maintain non-diabetic glucose levels. Some cases will go on to develop overt type 2 diabetes (154).

The present study classified TSH levels into three categories (<0.5, 0.5-1.5, >1.5 μIU/mL) and then assessed its relation with the studied biochemical parameters including glucose, total cholesterol, triacylglycerol, LDL and HDL. There was no significant relation between different levels of TSH and the studied parameters. However, the correlation statistics showed relatively weak negative correlation between TSH and total cholesterol and LDL levels. This negative correlation may be explained on the basis that increase in TSH may be due to increase in thyroid hormones which consequently decrease the concentrations of cholesterol, phospholipids and triacylglycerol in plasma(53). Similar results where obtained in obese individuals (163,164,165). Measurement of serum levels of TSH has been a consistent component of the clinical studies on the relationship between thyroid function and adiposity. The conclusion from some of these studies has been that weight gain increases serum levels of TSH; yet, others showed no relationship between TSH and body weight (166,167, 168).

Chapter 6

Conclusions and recommendations

6. Conclusion and recommendations

6.1 Conclusion

1. Obesity is a multifactorial disease associated with history of hyperlipidemia, weight gain and family history.

2. Getting fatigue quickly, muscle weakness and consumption of marine food, and large quantities of carbohydrates and lipids-rich food were related to obesity.

3. Physical activity particularly walking regularly and having active job protect against the development of obesity.

4. The mean levels of insulin and glucose were significantly increased in obese individuals compared to controls. In contrast, the mean levels of TSH were lower in obese individuals.

5. The mean levels of total cholesterol, triglycerides and LDL were increased in obese individuals compared to controls, whereas the mean level of HDL was significantly decreased in obese individuals.

6. No significant relation was found between the studied biochemical parameters including insulin, TSH, glucose, total cholesterol, triglyceride, LDL and HDL, and different grades of obesity.

7. The increases in the mean levels of insulin were significantly associated with increasing glucose levels .

8. No significant relation was found between the studied biochemical parameters and different TSH levels.

9. Insulin levels showed strong positive correlation with glucose level whereas TSH level showed relatively week negative correlations with total cholesterol and LDL levels.

6.2 Recommendations

1. Obese people are advisable to change their dietary lifestyle and habits**.**

2. Physical activity particularly walking and active lifestyle are recommended .

3. Insulin test is a very important test for obese individuals; this test may predict if the obese person is pre-diabetic or not. Other genetics tests are needed to ascertain insulin resistance.

4. Frequent monitoring of total cholesterol, triglyceride, LDL and HDL is recommended in obese individuals.

6. Health educational programs focusing on risk factors of obesity and its dangerous consequences are highly appreciated.

7. More research is needed to explore other risk factors which contributes to the development of obesity in our society.

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Appendices

Appendix 1

Questionnaire about, insulin, TSH and lipid levels among obese adult male in Gaza Governorate.

Demographic information:
Name:…… Age:……..
Occupation:……Address……
Marital status: single( ) married( ) others( )
Weight:……..height:…….BMI:…….
Education level……..
Phone number……

Question	Yes	No
1-have you been treated for thyroid problems in the past		
2-Do you get fatigued		
3-Do you have decreased sweating		
4-Do you suffer from muscle weakness		
5-Do you have slow heart rate		
6-Do you consume suitable quantities of any of the marine foods		
7-Do you have difficulty in concentrating or remembering things		
8-Do you sleep easily		
9-When you wake up do you feel lazy		
10-Is any of your relatives obese		
11-Do you eat carbohydrate or lipid (pie, cake, candy &ice cream) more than other food		
12- Do you eat just before bed time		
13- Do you snack between meals		
14-Do you have hyperlipidemia (high cholesterol or triglyceride)		
15-Do you gain weight inappropriately		
16-Do you try to lose weight but you fail		
17-Do you make exercise(any kind of games) regularly		
18-Do you walk regularly		
19-Does your job involve tasks that routinely keep you physically active		
20-Are you smoker		

استبانه حول العلاقة بين السمنة ومشاكل الغدة الدرقية مع قياس هرمون الأنسولين

الاسم.....العمر.....
العمل.....العنوان.....
الحالة الاجتماعية أعزب( ) متزوج( ) شيء أخر( )
الوزن.....الطول.....كتلة الجسم.....
المستوي التعليمي.....
رقم التلفون.....

السؤال	نعم	لا
- هل تعالجت لمشاكل بسبب الغدة الدرقية في الماضي		
2- هل تعاني من الإرهاق بشكل أسرع من المعتاد		
3- هل تشعر بنقص في الإفرازات العرقية		
4- هل تعانى من تعب عام في العضلات		
- هل تشعر ببطء في نبضات القلب		
- هل تتناول كميات كافية من المأكولات البحرية		
- هل تعانى من صعوبة في التركيز أو تذكر أشياء من الماضي		
8- عندما تذهب للفراش هل تنام بسهولة		
9- بعدما تاخد القدر الكافي من النوم هل تشعر بالكسل		
10- هل لديك أقارب يعانون من مشكلة السمنة		
11- هل تتناول كمية عالية من النشويات والدهون (كيك ،معجنات، فطائر،مكسرات،شيكولاتة،بوظة		
12- هل تتناول وجبة طعام قبل النوم مباشرة		
13- هل تتناول وجبات خفيفة بين الوجبات الرئيسية		
14- هل تعانى من مشكلة ارتفاع نسبة الدهون في الدم -الكولسترول أو الدهون الثلاثية		
15- هل تشعر أن وزنك يزداد بشكل لافت للنظر		
16- هل حاولت إنقاص وزنك - رجيم- وفشلت في ذلك		
17-هل تمارس الرياضة(أي نوع من الالعاب) بشكل منتظم		
18- هل تمارس رياضة المشي بشكل منتظم		
19- هل طبيعة عملك تتطلب حركة مستمرة		
20- هل انت مدخن		