Sudan University of Science and Technology

College of Graduate Studies

The Impact of Ketogenic Diet on Body weight and Insulin Resistance Index among Sudanese Obese Females

تأثير النظام الغذائي الكيتوني على وزن الجسم ومقاومة الإنسولين لدى الإناث السودانيات البدينات

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الآية

اللَّهُ نُورُ السَّمَاوَاتِ وَالْأَرْضِ ۚ مَثَلُ نُورِهِ كَمِشْكَاةٍ فِيهَا مِصْبَاحٌ ۖ الْمِصْبَاحُ فِي زُجَاجَةٍ ۖ الزُّجَاجَةُ كَأَنَّهَا كَوْكَبٌ دُرِّيٌّ يُوقَدُ مِنْ شَجَرَةٍ مُبَارَكَةٍ زَيْتُونَةٍ لَا شَرْقِيَّةٍ وَلَا غَرْبِيَّةٍ يَكَادُ زَيْتُهَا يُضِيءُ وَلَوْ لَمْ تَمْسَسْهُ نَارٌ ۚ نُورٌ على نُورٍ ۗ يَهْدِي اللَّهُ لِنُورِهِ مَنْ يَشَاءُ ۚ وَيَضْرِبُ اللَّهُ الْأَمْثَالَ لِلنَّاسِ ۗ وَاللَّهُ بِكُلِّ شَيْءٍ عَلِيمٌ ﴿٣٥﴾

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Dedication

This study is wholeheartedly dedicated to my beloved parents who have been my source of inspiration and gave me strength when I thought of giving up, who continually provide their moral spiritual and emotional support.

To my siblings and my friends who have always been a constant source of support and encouragement during all the challenges.

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Abstract

The persistence of an epidemic of obesity and type 2 diabetes suggests that new nutritional strategies are needed. This quasi study was carried out to investigate the impact of ketogenic diet on body weight and insulin resistance index among Sudanese obese females in Khartoum state from September 2019 to December 2019.

A total of 20 participants were included in this study, twenty blood samples were collected from the obese females before starting the diet, All 20 subjects were instructed to follow a ketogenic diet consisting of less than 5% of carbohydrates and 25% of proteins and 70% of fat. Only 15 participants completed 12 weeks successfully, other samples were collected from each.

Weight measurement was took every two weeks. The estimation of fasting blood glucose, and insulin level was done by using COBUS c111 and TOSOH 1800 respectively, and results were analyzed by using SPSS computer program. Statistical analysis showed a significant decrease in insulin resistance index when compared before and after the diet (mean 2.5±1.3, 1.5±0.9, P.value= 0.00 respectively). Moreover, a significant reduction in fasting blood glucose level when compared the level before and after the diet (mean 87.8±6.4, 80.2±7.2 mg/dl, P.value= 0.00 respectively).

Also showed a significant decrease in fasting insulin level when compared the level before and after the diet (mean 11.5±6, 7.4±4.8 μU/ml, P.value= 0.00 respectively).And a significant decrease in the weight when compared to the weight before and after the diet (mean 101.3±10.3, 90.3±8.6 Kg, P.value= 0.00 respectively).

Statistical analysis also showed a significant reduction in BMI when compared to the BMI before and after the diet (mean 36.5±3.9, 32.5±3.2 kg/m², P.value= 0.00 respectively).

The analysis revealed that the differences of weight between all combinations of related groups is not equal, (P.value = 0.00), and there was a statistically significant difference between the means of weight at the different points of time, (P.value= 0.00).

The results also showed a significant difference in weight loss between all weeks (P.value is < 0.005) except for week 4 and week 6, there was no significant differences (P.value = 0.095).

The analysis showed no significant correlation between age and Insulin resistance before and after the ketogenic diet, (P-value = 0.804,
0.605, respectively).

Conclusion: There was decrease of insulin resistance index, weight and BMI among Sudanese obese females after following the ketogenic diet for three months. Therefore ketogenic diet has a positive impact on body weight and insulin resistance index.

مستخلص الدراسة

يشير استمرار معدل انتشار السمنة ومرض السكري من النوع الثاني إلى الحاجة إلى استراتيجيات غذائية جديدة للتغلب على السمنة ومضاعفاتها، وقد أجريت هذه الدراسة شبه التجريبية لبحث تأثير النظام الغذائي الكيتوني على وزن الجسم ومؤشر مقاومة الإنسولين بين الإناث السودانيات البدينات في ولاية الخرطوم، من سبتمبر 2019 إلى ديسمبر 2019.

تم تضمين عشرون مشتركة في هذه الدراسة، وجمع عشرين عينة دم من الاناث البدينات قبل بدء النظام الغذائي، وتم توجيه جميع المشاركات في الدراسة باتباع نظام غذائي كيتوني يتكون من أقل من 5٪ من الكربوهيدرات و25٪ من البروتينات و70٪. من الدهون. أكملت 15 مشاركة فقط مدة الدراسة (12 أسبوعًا) بنجاح، و الإنسولين تم جمع عينة أخرى من كل منهم.

تم أخذ قياسات الوزن كل أسبوعين ثم تم تقدير سكر الدم ومستوى الأنسولين في حالة الصيام ثم تحليل النتائج احصائيا باستخدام برنامج التحليل الاحصائي. أظهر التحليل الإحصائي انخفاضًا ذو دلالة احصائية في مقاومة الإنسولين عند مقارنته قبل وبعد النظام الغذائي (2.5 ± 1.3، 1.5 ± 0.9، على التوالي) القيمة الاحتمالية(0.00).

بالإضافة الى انخفاض في مستوى الجلوكوز في الدم مقارنة بالمستوى قبل وبعد اتباع النظام الغذائي (87.8 ± 6.4، 80.2 ± 7.2 ملغ / ديسيلتر، على التوالي) القيمة الاحتمالية(0.00).

كذلك انخفاض ذو دلالة احصائية في مستوى الإنسولين عند مقارنته بالمستوى قبل وبعد النظام الغذائي (11.5 ± 6، 7.4 ± 4.8 ميكرو وحدة/ملليتر، على التوالي) القيمة الاحتمالية(0.00).

وانخفاض في الوزن عند مقارنته قبل وبعد النظام الغذائي (101.3 ± 10.3، 90.3 ± 8.6 كيلوجرام، على التوالي) القيمة الاحتمالية(0.00).

كما أظهر التحليل الإحصائي أيضًا انخفاضًا كبيرًا في مؤشر كتلة الجسم عند مقارنته بمؤشر كتلة الجسم قبل وبعد النظام الغذائي (36.5 ± 3.9، 32.5 ± 3.2، كجم/م^2 قيمة التوالي) القيمة الاحتمالية(0.00).

وأظهر التحليل الإحصائي أن فرق الوزن بين كل المجموعات المترابطة ليست متساوية باعتبار كل فترة زمنية تشير إلى مجموعة، القيمة الاحتمالية (0.00)، وكان هناك فرق ذو دلالة إحصائية بين متوسطات الوزن في فترات زمنية مختلفة، القيمة الاحتمالية (0.00).

كما أوضحت النتائج وجود فرق ذو دلالة احصائية في فقدان الوزن بين جميع الأسابيع (القيمة الاحتمالية <0.005) باستثناء الأسبوع الرابع والأسبوع السادس، حيث لم تكن هناك فروق ذات دلالة احصائية (القيمة الاحتمالية 0.095).

أظهر التحليل الإحصائي أيضا عدم وجود علاقة ذات دلالة احصائية بين العمر ومقاومة الأنسولين قبل وبعد النظام الغذائي الكيتوني، (القيمة الاحتمالية = 0.804، 0.605 على التوالي).

الخلاصة: كان هناك انخفاض في مؤشر مقاومة الأنسولين والوزن ومؤشر كتلة الجسم بين الإناث السودانيات البدينات بعد اتباع النظام الغذائي الكيتوني لمدة ثلاثة أشهر. لذلك فإن النظام الغذائي الكيتوني له تأثير إيجابي على وزن الجسم ومؤشر مقاومة الأنسولين.

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List of Abbreviations

Abbreviation	Stand for
ADP	Adenosine diphosphosphate
AEDs	antiepileptic drugs
ALS	Amyotrophic lateral sclerosis
ATP	Adenosine triphosphosphate
BHB	B-hydroxybutyrate
BMI	Body mass index
BMR	basal metabolic rate
CHD	Coronary heart disease
CVD	Cardiovascular disease
DM	Diabetes mellitus
FFAs	FREE FATTY ACIDS
GIR	glucose infusion rate
HDL	High density lipoprotein
HIEG	hyperinsulinaemic–euglycaemic glucose
HOMA	Homeostatic model assessment
IL-6	Interleukin 6
IR	Insulin resistance
IST	Insulin suppression test
KATP	ATP-sensitive K channels
KD	Ketogenic diet
LDL	Low density lipoprotein
NAFLD	non-alcoholic fatty liver disease
OGTT	Oral glucose tolerance test

OSA	obstructive sleep apnea
PCOS	Polycystic ovary syndrome
PPAR-y	gamma receptor activating peroxisome proliferator
QUICKI	quantitative insulin sensitivity check index
RER	rough endoplasmic reticulum
SPSS	Statistical package for social sciences
SSPG	Steady-State Plasma Glucose
WC	Waist circumference

1. Introduction

1.1 Introduction

The global epidemic of overweight and obesity has grown and has become a major health issue. According to recent estimates, more than 600 million people worldwide are reportedly affected by obesity, and it has also characterized by defects of insulin action (Lois and Kumar, 2009).

As a common disease in obesity and type 2 diabetes, insulin resistance is characterized as a condition in which cells fail to respond to insulin, leading to the development of hyperglycemia (Su *et al.*, 2019).

Insulin resistance seen in obesity is thought to mainly include muscle and liver, with increased adipocyte-derived free fatty acids promoting triglyceride accumulation in these tissues(Perseghin *et al.*, 2003). Due to the obesity epidemic, there has been a rise in the public interest and use of alternative weight loss diets that contravene traditional dietary guidelines. Popular alternatives are very low carbohydrate diets, especially ketogenic diets, which have a common theme of limiting carbohydrate intake while increasing fat and protein intake (Astrup, Meinert Larsen and Harper, 2004).

The ketogenic diet (KD) is a low-carbohydrate, high-fat diet that is used for a variety of health-related effects (Hartman and Vining, 2007). This type of diet is effective in suppressing seizure activity in children with refractory epilepsy and may have been more commonly implemented as a dietary strategy whereby weight maintenance or weight loss is the desired outcome (Kinzig, Honors and Hargrave, 2010).

Dietary carbohydrates and insulin have been proposed to play a causal role in the pathological accumulation of body fat. According to this, an increased proportion of the diet as carbohydrates results in increased insulin secretion, which suppresses the release of fatty acids into circulation and guides the flow of fat to storage. In addition, reduced supply of fatty acids for use in metabolically active tissues, such as the heart,

muscle, and liver, is viewed as a state of internal cell starvation (Hall *et al.*, 2018).

Several studies have shown that a short-term ketogenic diet can be helpful in obtaining a quick and relatively safe weight. In addition, the efficacy of body weight loss appears to be associated with a large number of positive metabolic changes, potentially useful for alleviating the characteristics of metabolic syndrome and contrasting the development of type 2 diabetes. In fact, a recent meta-analysis of randomized clinical trials found that ketogenic diets lead to a longer-term, more significant improvement in body weight, diastolic blood pressure, triglycerides and HDL-cholesterol compared to low-fat diets (Cicero *et al.*, 2015).

However, the impact of the ketogenic diet on insulin resistance and glucose metabolism are still under debate.

1.2 Rationale

Obesity is implicated as a cause of many debilitating diseases, including diabetes, cardiovascular disorders, and cancers. Insulin resistance is a pathological condition closely associated with obesity (Kosinski and Jornayvaz, 2017).

People who develop type 2 diabetes typically go through earlier stages, and this does not happen there is current failure of compensatory insulin secretion (Wang, 2014).

Recent studies have demonstrated the beneficial effects of ketogenic diet in overweight and obese patients. It has been shown that, in addition to reducing the weight of overweight and obese individuals, it reduces risk factors for cardiovascular disease (Talib *et al.*, 2007).

Many studies were conducted to estimate the impact of ketogenic diet on body weight and insulin resistance among obese individuals, but in Sudan, no published studies were found, although there is increase prevalence of obesity and popularity of ketogenic diet, that is why I attempted to do this study.

1.3 Objectives

1.3.1 General objectives:

To study the impact of ketogenic diet on body weight and insulin resistance index among obese Sudanese females from September 2019 to December 2019.

1.3.2 Specific Objectives:

1- To estimate fasting insulin level and fasting blood glucose in obese females before and after the ketogenic diet.

2- To measure the weight differences over twelve weeks.

3- To compare insulin resistance and BMI before and after the diet.

Chapter Two
Literature Review

2. Literature review

2.1 Ketogenic Diet

"Ketogenic diet is a high fat, low carbohydrate, normocaloric diet that mimics the metabolic state of fasting. During a prolonged fast, body energy requirements are met by lipolysis and β-oxidation of fatty acids rather than by the breakdown of carbohydrates". Thus, in a metabolic situation of fasting the KD retains an anabolic nutritional state. Any diet that provides dietary fat for ketone generation that serves as an alternative fuel to body tissues may be considered "ketogenic"(Freeman *et al.*, 2006).

2.1.1 Early history

The practice of managing seizures by dietary therapy has persisted throughout much of the literature known, but in 1911, a pair of Parisian physicians Gulep and Marie documented the first modern use of starvation as medication for epilepsy, they treated 20 children and adults with epilepsy, and confirmed that seizures were less serious after treatment (Höhn *et al*, 2019).

A diet consisting mostly of fats, i.e. a high-fat, low-carbohydrate ' ketogenic diet, ' was found to be capable of replicating the effects of fasting, and these beneficial effects were attributed to the production of ketones, such as b-hydroxybutyrate (BHB), acetoacetate, and acetone in the liver (Freeman, 2010).

Despite early results in ketogenic diet therapy, the development of antiepileptic drugs (AEDs) in the 1940s moved ketogenic diet therapy to the sidelines. Nevertheless, the therapeutic use of ketogenic diet therapy gained increased attention in the 1990s, and ketogenic diet therapies are now known for complicated epilepsy treatment in addition to broader use in a range of neurological disorders (Talib *et al.*, 2007).

2.1.2 Physiology and Biochemistry

Carbohydrates are the primary source of energy for body tissue growth. When the body is deprived of carbohydrates due to lower intake to less than 50 g per day, insulin production is significantly reduced and the body enters a catabolic condition. Glycogen stores are depleting, causing the body to undergo certain metabolic changes. Two metabolic processes occur when the concentration of carbohydrates in body tissues is low: gluconeogenesis and ketogenesis (Jagadish *et al.*, 2019).

Gluconeogenesis is the endogenous production of glucose in the body, especially in the liver primarily from lactic acid, glycerol, and the amino acids alanine and glutamine. As glucose supply continues to decline, endogenous glucose production is not able to meet the needs of the body, and ketogenesis starts to provide an alternative energy source in the form of ketone bodies. Ketone bodies substitute glucose as the primary energy source. During ketogenesis due to low blood glucose feedback insulin secretion stimulation is also limited, which significantly reduces the signal for fat and glucose storage. Other hormonal changes may contribute to an increased breakdown of fats resulting in fatty acids. Fatty acids are metabolized to acetoacetate, which is then converted to beta-hydroxybutyrate and acetone. These are the main ketone bodies that accumulate in the body as a ketogenic diet is maintained. This metabolic condition is referred to as "nutritional ketosis." While long as the body is deprived of carbohydrates, the metabolism remains ketotic state. The nutritional ketosis state is known to be quite safe as ketone bodies are released in small concentrations without any alteration in the pH of the blood. This differs greatly from ketoacidosis, a life-threatening condition in which ketone bodies are formed at extremely high concentrations, altering blood PH to acidotic a state.

Ketone body synthesized in the body can easily be used to produce energy from the heart, muscle tissue, and kidneys. Ketone bodies can also cross the blood-brain barrier to provide the brain with an alternate source of energy. RBCs do not use ketones due to lack of mitochondria. Ketone body production depends on several factors such as resting basal metabolic rate (BMR), body mass index (BMI), and body fat percentage. Ketone bodies produce more adenosine triphosphate in comparison to glucose, sometimes aptly called a "super fuel." One hundred grams of acetoacetate generates 9400 grams of ATP, and 100 g of beta-hydroxybutyrate yields 10,500 grams of ATP; whereas, 100 grams of glucose produces only 8,700 grams of ATP It allows the body to sustain efficient fuel output even in the presence of a caloric deficit. Ketone bodies also minimize free radical damage and enhance antioxidant efficiency (Masood W, 2019).

2.1.3 Ketogenic diet and weight loss

There is no doubt that there is strong supporting evidence that the use of ketogenic diets in weight loss therapy is successful. However, the mechanisms underlying the effects of KDs on weight loss are still under discussion. Atkins ' original hypothesis suggested that weight loss was caused by the loss of energy by the excretion of ketone bodies.

During the first phase of KD, 60–65 g of glucose per day is required by the body, 16% of which is generated from glycerol, while the major part is produced from proteins, either dietary or tissue, through gluconeogenesis (Westerterp-Plantenga *et al.*, 2009).

Gluconeogenesis is an energy-demanding mechanism estimated at approximately 400–600 Kcal / day (due to both endogenous and food-source proteins). However, there is no clear experimental evidence to support this intriguing hypothesis; on the contrary, a recent study recorded that there were no changes in resting energy expenditure after KD (Paoli *et al.*, 2012).

Some authors claim instead that the results obtained with ketogenic diets could be attributed to a reduction in appetite due to higher satiety effect of proteins or to some effects on appetite control hormones (Sumithran, *et al*, 2013). Other authors suggest a possible direct appetite suppressant action of the ketone bodies. and more in detail by the BHB that it is supposed to act both as an energy/satiety signal (according to Kennedy's lipostatic theory) and as central satiety signal.(Laeger, Metges and Kuhla, 2010) Over the long term, the improvement in fat oxidation mirrored by the decrease of RR (respiratory ratio) could explain the fat loss effect of this kind of diet (Paoli *et al.*, 2012).

2.1.4 Effects of ketogenic diet

It could be claimed that the ketogenic diet has beneficial effects other than mere fat and weight loss. Beyond reducing weight, low- carbohydrate diets can also help improve blood pressure, controlling blood glucose, triglycerides, and cholesterol levels in HDL. However, LDL cholesterol may increase on this diet (Kosinski and Jornayvaz, 2017).

In addition, in various studies, the ketogenic diet has shown promising results in a variety of neurological disorders, like epilepsy, dementia, ALS, traumatic brain injury, acne, cancers, and metabolic disorders (Armeno *et al.*, 2014).

While the ketogenic diet can help one lose weight in the short term, that is not maintained in the long run. In fact, numerous studies indicate that the diet is associated with many complications that often lead to visits and admissions to emergency rooms for fatigue, electrolyte deficiencies and hypoglycemia (Kang *et al.*, 2004).

2.1.5 Background studies

A study conducted by Bisschop et al showed that a high-fat, low-carbohydrate ketogenic diet reduces the ability of insulin to suppress endogenous glucose production in healthy men, Six healthy men were

studied for 11 days diet Insulin sensitivity was quantified by using the gold standard method, the hyperinsulinemic-euglycemic clamp (Bisschop *et al.*, 2001).

Other study described the opposite: with a KD, 132 severely obese subjects (including 77 men and 23 women) non-diabetic participants had a significant lower HOMA-IR and fasting glucose after a five months KD than at baseline and an improvement in insulin sensitivity using the QUICKI (Samaha *et al.*, 2003a).

Finally, another trial involving 63 obese men and women, Plasma insulin was measured by radioimmunoassay, and plasma glucose by a glucose oxidase autoanalyzer (Yellow Springs Instruments) the result described a better insulin sensitivity by consuming a KD, but this improvement does not seem to be permanent. Indeed, Forster et al. found a significant improvement in insulin sensitivity at 6 months, but not at 1 year (Foster *et al.*, 2003).

2.2 Obesity

Obesity is a medical condition in which excess body fat has accumulated to the level that it may have a detrimental effect on health, leading to a decrease in life expectancy and/or increased health issues. People are considered obese when their Body Mass Index (BMI), a measure that compares their weight and square height, exceeds 30 kg / m2. (Khan *et al.*, 2012).

According to Keaver et al., overweight and obesity was expected to exceed 89% and 85% for males and females by 2030, respectively. This will result in an increase of 97% in the incidence of obesity-related coronary heart disease (CHD), 61% in cancers and 21% in type 2 diabetes.(Keaver *et al.*, 2013).

2.2.1. Classification of obesity according to body mass index (BMI):

Obesity is usually classified by BMI. It is calculated as body weight in kilograms divided by the height in meters squared (kg/m2).

BMI is usually expressed in kilograms per square meter. To convert from pounds per square inch multiply by 703 (kg/m2)/(lb/sq).(Aronne, 2002).

Other methods including waist circumference (WC) and central and peripheral fat mass, have also been used, but currently BMI is continued to be used for the classification of obesity.

World health organization provides the values listed in the table below:

Table 2.1: The International Classification of adult underweight, Overweight and obesity according to BMI (World Health Organization, 2000).

Classification	BMI(kg/m2)
Underweight	<18.5
Normal	18.5-24.9
Overweight	25.0-29.9
Obesity class I	30.0-34.9
Obesity class II	35.0-39.9
Obesity class III	≥40.00

2.2.2 Causes of obesity:

While obesity is most commonly caused by excess energy consumption (dietary intake) relative to energy expenditure (energy loss through metabolic and physical activity), the etiology of obesity is highly complex and involves genetic, physiological, environmental, psychological and even economic factors that interact to varying degrees to contribute to the development of obesity (Wright and Aronne, 2012).

In general, the principal causes of obesity are:

2.2.2.1 Overeating. Overeating is often a difficult habit to break between the overweight and the obese. Not only that, those who are obese tend to eat faster, there is a correlation between behavior and body weight, because the faster they eat, the more full signals can be missed, whether intentionally or not. The increased use of high-fructose sugar-flavored soft drinks is a prime suspect in the increase in obesity (Anderson and Matsa, 2011).

2.2.2.2 Physical activity. The level of physical activity is also important. Daily activities now require so little effort that voluntary physical activity is a key determinant of energy needs. In addition, cross-sectional studies have often established correlations between leisure-time physical activity

(inverse) and total time spent sitting (direct) and BMI, while low participation in sports activities, lack of interest in exercise and a high number of hours spent sitting at work are statistically significant predictors of obesity (Martínez-González *et al.*, 1999).

Physical activity provides significant health benefits, regardless of body weight, in particular by helping to maintain insulin sensitivity and reduce the risk of type 2 diabetes.

2.2.2.3 Genes. A small number of cases of obesity are attributed to genetic factors, contributing to particular clinical syndromes. Monogenic causes of obesity include mutations in the leptin gene and its receptor or in the melanocortin system. Other genetic syndromes occur in which obesity is a common element of the phenotype (e.g. Prader–Willi, Bardet–Biedl). Furthermore, they account for only a small proportion of the total cases of obesity and usually show a marked obesity from a relatively early age. A large number of other candidate genes have been associated with obesity. Heritability studies suggest that approximately one third of the variance in BMI may be due to genetic factors. The potential for some behavioral traits to have a genetic component is increasingly recognized, such as food preferences or exercise habits.(Martinez, 2000).

2.2.2.4 Drugs. The deleterious effects of drug-induced weight gain include, paradoxically, increased risks for developing type II diabetes, hypertension, hyperlipidemia, as well as poor medication compliance. While it is difficult to estimate the full impact of drug-induced weight gain, the recognition that some of the most widely prescribed classes of drugs can cause significant weight gain supports the hypothesis that drug-induced weight gain is contributing to the obesity epidemic.(Wright and Aronne, 2012).

2.2.2.5 Socio-economic factors. Obesity is not just an individual concern. The increase in obesity due to economic development suggests that there is a latent susceptibility to obesity. Around the world there are examples of communities following traditional lifestyles who, when exposed to 21st century Western culture, rapidly gain weight and develop diabetes. However, overweight in families, tends to be the result of shared genes and a shared family lifestyle. Social change means greater dependence on convenience foods and more food consumed outside the home; these tend to be higher in fat and sugar than home-made food. (Jebb, 2004).

Moreover, minority population groups had less access to physical activity services, which is associated with reduced physical activity and increased overweight.

Higher rates of obesity are likely to be among the lowest income and the least educated, especially among women and certain ethnic groups. The correlation between hunger and obesity can be explained by the relatively low cost of energy-dense food, the high taste of sweets and fats associated with higher energy intakes, and the association of lower income and food insecurity with lower intakes of fruit and vegetables.

Studies have found that healthier food is generally more costly and less readily accessible in poorer communities.(Akil and Ahmad, 2011)

2.2.3 Consequences of Obesity:

Major obesity-related diseases include hypertension, atherosclerosis, and diabetes, as well as certain types of cancer. less documented complications include hepatic steatosis, gallbladder disease, impaired pulmonary function, endocrine anomalies, obstetric complications, weight-bearing joint trauma, gout, skin disease, proteinuria, elevated concentration of hemoglobin, and, and possibly immunologic impairment (Peeters *et al.*, 2003) .

These effects of obesity are attributed to two factors: increased mass of adipose tissue and increased secretion of pathogenic products from enlarged fat cells. The definition of obesity pathogenesis as a disease allows for easy separation of obesity drawbacks between those created by fat mass and those produced by the metabolic effects of fat cells. In the former category are the social disabilities resulting from the stigma associated with obesity, sleep apnea that results in part from increased parapharyngeal fat deposits, and osteoarthritis resulting from the wear and tear on joints from carrying an increased mass of fat. The second category concerns the metabolic factors associated with the distant effects of products released from enlarged fat cells. The insulin-resistant condition so prevalent in obesity is likely to reflect the effects of increased release of fatty acids from fat cells, which are then retained in the liver or muscle. When the fight against insulin resistance overwhelms the secretive capacity of the pancreas, diabetes develops.

The strong association of increased fat, especially visceral fat, with diabetes makes this particularly ominous for health care costs. The release of cytokines, especially IL-6, from the fat cell that stimulate the proinflammatory state that characterizes obesity. Increased secretion of the fat-cell prothrombin activator-1 receptor may play a role in the procoagulant state of obesity and may along with changes in endothelial function, be responsible for increased risk of cardiovascular disease and hypertension. In the case of cancer, the development of estrogens by increased stromal mass plays a role in the risk of breast cancer.

Increased release of cytokine may play a role in other forms of proliferative growth. The combined effect of these pathogenic consequences of increased fat storage is an increased risk of shortening life expectancy (Bray, 2004).

2.2.4 Management of obesity

2.1.4.1 Dietary Therapy. In most overweight and obese patients, it will be necessary to adjust the diet to reduce caloric intake. Dietary therapy involves, in large part, teaching patients how to modify their diets in order to achieve a reduction in caloric intake. The use of a gradual reduction of caloric intake to achieve a steady but successful weight loss is a key element of the current recommendation. Ideally, caloric intake should only be limited to the amount required to maintain weight at the desired level. If this amount of caloric intake is met, the excess weight will gradually disappear. For practice, slightly higher caloric deficits are used during the successful weight loss period, but diets with very low calories are avoided. Finally, the composition of the diet should be adjusted to reduce other cardiovascular risk factors.

2.2.4.2 Physical Activity. Increasing physical activity is an important component of weight loss therapy as it leads to increased energy expenditure. Increased physical activity may also inhibit the intake of food in overweight patients. Physical activity may also be helpful in maintaining a desirable weight. In fact, sustained physical activity has the benefit of reducing the overall risk of CHD beyond that of weight reduction alone.

2.2.4.3 Behavior Therapy. The goal of behavioral therapy is to change the eating and working habits of obese patients. Behavioral strategies to strengthen dietary changes and physical activity can result in weight loss in obese adults ranging from 10% of baseline weight over 4 months to 1 year. Unless a patient has acquired a new set of eating and physical activity habits, long-term weight reduction is unlikely to succeed. Acquiring new habits is particularly important for maintaining long-term weight at a lower weight (Donato, 2007).

2.3 Insulin

Insulin is a peptide hormone secreted by the β cells of the pancreatic islets of Langerhans and maintains normal blood glucose by encouraging the absorption of cellular glucose, regulating the metabolism of carbohydrates, lipids and proteins (Wilcox, 2005).

2.3.1 Structure and Chemical Properties of Insulin:

Insulin was discovered to be a polypeptide in 1928 with an amino acid sequence described in 1952. In fact, it is a dipeptide, containing chains A and B, bound by disulphide bridges and containing 51 amino acids, with a molecular weight of 5802.Its iso-electric point is pH 5.5.

The A chain consists of 21 amino acids and the B chain contains 30 amino acids. The A chain has an N-terminal helix connected to the anti-parallel C terminal helix; the B chain has a central helix section. The two chains are linked by two disulphide bonds, which bind the N-and C-terminal helices of the A chain to the central helix of the B chain. In pro-insulin, a connecting peptide links the N-terminus of the A chain to the C-terminus of the B chain (Dodson and Steiner, 1998) .

2.3.2 Insulin synthesis:

Insulin is encoded on the short arm of chromosome 11 and synthesized as its predecessor, proinsulin, in the β cells of the pancreatic islets of Langherhans. Proinsulin is synthesized as pre-proinsulin in ribosomes of the rough endoplasmic reticulum (RER) of mRNA. Pre-proinsulin is produced by sequential synthesis of the signal peptide, the B-chain, the connecting (C) peptide, and then the A-chain consisting of a single chain of 100 amino acids. Removal of the signal peptide forms proinsulin, which acquires its characteristic three-dimensional structure in the endoplasmic reticulum. Secretory vesicles pass proinsulin from the RER to the Golgi apparatus, whose aqueous zinc and rich calcium condition promote the formation of soluble zinc-containing proinsulin hexamers.

When immature storage vesicles emerge from Golgi, enzymes acting outside Golgi convert proinsulin to insulin and C to peptide. Insulin forms zinc-containing hexamers that are insoluble and precipitate when chemically stable crystals at pH 5.5. Once mature granules are secreted into circulation by exocytosis, insulin and the equimolar C-peptide ratio are released. Proinsulin and zinc usually make up no more than 6% of the secretion of the islet cells (Malaisse *et al.*, 1997).

2.3.3 Insulin Secretion

β -cells are excitable endocrine cells that secrete insulin. Glucose-stimulated, KATP channel dependent pathway starts with intracellular transport of extracellular glucose by glucose transporter (GLUT2). Intracellular glucose undergoes cytosolic glycolysis catalyzed by glucokinase. Pyruvate, a product of glycolysis, is shuttled into mitochondria as a substrate of the tricarboxylic acid cycle with production of adenosine triphosphate (ATP). As a result, cytosolic ATP levels are elevated and adenosine 5´-diphosphate (ADP) levels reduced. Increased cytosolic ATP/ADP ratio closes an ATP-sensitive K+ channel, KATP, which results in discontinued K+ outflow, thus depolarizing the β.cell membrane. Voltage-dependent calcium channels (VDCCs) on the cell membrane are opened by depolarization, and calcium influx increases intracellular calcium (phase1), which, in turn, activates calcium-dependent calcium release from the endoplasmic reticulum (phase 2).

The resultant biphasic increase in intracellular calcium triggers fusion of secretory vesicles with the plasma membrane and insulin is released. The KATP channel plays the crucial role of converting metabolic to electric signals. All components of glucose- stimulated, KATP channel-dependent insulin secretion are found in several other cell types, except for the KATP channel, which is unique for β cells (a similar channel is also found in muscle and brain) (Bonner-Weir *et al.*, 2000).

Factors Influencing Insulin Biosynthesis and Release Insulin Secretion may be affected by changes in synthesis at the Golgi gene transcription, translation, and post-translational alteration stages, as well as by factors affecting insulin release from secretory granules.(Bratanova-Tochkova *et al.*, 2002).

2.3.4 Insulin disturbance is pathologic in several conditions:

Diabetes mellitus: Systemic metabolic disorder characterized by a tendency to chronic hyperglycaemia with carbohydrate, fat and protein metabolism disorders that result from an insulin secretion or action defect or both.

There are two distinct types in type 1 DM; there is destruction of pancreatic cells, leading to a decrease in, and eventually cessation of, insulin secretion. Approximately 10% of all patients with diabetes have type 1. They have an absolute requirement for insulin. During type 2 DM, insulin secretion is deficient and delayed and there is resistance to its action. Many patients with type 2 DM may initially be treated successfully by diet, with or without oral hypoglycaemic drugs, but many will eventually require insulin therapy to maintain sufficient glycemic control.

Insulinoma: Insulinomas are tumors of the insulin-secreting β-cells of the pancreatic islets (Marshall, *et al* 2012).

Metabolic syndrome: Also labeled as' insulin resistance syndrome,"x syndrome' and' hypertriglyceridemic waist,' a cluster of metabolic abnormalities includes hypertension, central obesity, insulin resistance, and atherogenic dyslipidemia. Metabolic syndrome is strongly linked to an increased risk of developing atherosclerotic cardiovascular disease.

The pathogenesis of Metabolic Syndrome involves both genetic and acquired factors that play a role in the final pathway of inflammation that leads to CVD.

The underlying cause may be insulin resistance that precedes type 2 diabetes, which is a reduced insulin response ability in some tissues (e.g. muscle, fat). Metabolic Syndrome has become increasingly relevant in recent times due to an unprecedented increase in global obesity (Rochlani, Pothineni and Kovelamudi, 2017).

2.4 Insulin Resistance (IR)

Insulin resistance is defined clinically as the inability of a known quantity of exogenous or endogenous insulin to increase glucose uptake and utilization in an individual as much as it does in a normal population (Lebovitz, 2001).

Insulin resistance is mainly an acquired disorder attributable to excess body fat, although genetic factors are also known. Metabolic effects of insulin resistance may result in hyperglycemia, hypertension, dyslipidemia, visceral adiposity, hyperuricemia, elevated inflammatory markers, endothelial dysfunction, and prothrombosis. Insulin resistance progression can cause metabolic syndrome, non-alcoholic fatty liver disease (NAFLD) and type 2 diabetes mellitus (Hossain *et al.*, 2019).

2.4.1 Pathophysiology of insulin resistance

The muscle, liver and adipose tissue are the three primary sites of insulin resistance. Insulin resistance is postulated to begin with immune-mediated inflammatory shift and excess free fatty acids in the muscle tissue, causing deposition of ectopic lipids. Muscle accounts for up to 70% of glucose disposal. Excess glucose returns to the liver with impaired muscle absorption, increasing de novo lipogenesis (DNL) and circulating free fatty acids, further leading to ectopic fat deposition and insulin resistance (Freeman AM, 2019).

Adipose Tissue

Researchers have determined that lipolysis is most sensitive to insulin by using the hyperinsulinemic-euglycemic clamp technique. Insulin failure to suppress lipolysis in insulin-resistant adipose tissue, especially visceral adipose tissue, increases the fatty acids that circulate freely. Higher levels of circulating FFAs directly affect the metabolism of the liver and muscle, exacerbating insulin resistance even further.

Muscle Tissue

Muscle is the primary site for glucose disposal after intake of a caloric load and conversion to glucose, representing up to 70 percent of tissue glucose uptake. Glucose absorption by muscle exceeds capacity with excess calorie loads, and excess glucose returns to the liver where it causes DNL.

Increased DNL lead to more production of triglyceride and FFA, causing deposition of ectopic fat into the liver, muscle, and adipose tissue. As a result, both the insulin resistance and the development of inflammatory markers are increasing. Physical inactivity and genetic risk are additional factors affecting insulin resistance in muscle tissue.

Hepatic Tissue

Insulin resistance in the muscle results in increased delivery of glucose substrate to the liver, causing DNL, associated with inflammation, and deposition of ectopic lipids. Insulin resistance in adipose tissue leads to increased lipolysis in adipocytes, resulting in increased secretion of FFA and increased steatosis and insulin resistance in the muscle tissue. In the presence of caloric intake, insulin decreases the production of hepatic glucose through glycogenolysis inhibition, thereby reducing the postprandial increase in glucose. This feedback mechanism is compromised with insulin resistance, and development of hepatic glucose continues to rise, even as postprandial glucose increases. Glucotoxicity,

associated with elevated glucose levels, further contributes to insulin resistance (Wilcox, 2005).

2.4.2 Causes of insulin resistance

There are many factors lead to development of insulin resistance. The major leading cause are obesity and fat distribution centrally positioned adipose tissue (abdomen and waist) particularly in the viscera is involved in insulin resistance. Two molecular peptides-Leptin and Adiponectin-are also suspected.

Age. Increase in age reduces insulin sensitivity.

Genetics. Numerous gene mutations, which are not well known, have been linked to insulin resistance. The few known are rare and less than 4%. Example is the gamma receptor activating peroxisome proliferator (PPAR-y). The genetic predisposition begins with beta-cell dysfunction from gestation through early years to adulthood (Nolan, Damm and Prentki, 2011).

Other factors that may affect the degree of insulin resistance are dietary composition, physical inactivity, and hormones (especially glucocorticoids and androgens). High carbohydrate diets replicate some of the metabolic syndrome's symptoms (Alborhan, 2015).

2.4.3 Signs and symptoms of insulin resistance

- Brain fogginess and inability to focus.
- High blood sugar.
- Intestinal bloating – most intestinal gas is produced from carbohydrates in sleepiness, especially after meals.
- Weight gain, fat storage, difficulty-losing weight for most people, excess Weight is from high fat storage; the fat in IR is generally stored in and around abdominal organs in both males and females; it is currently

suspected that hormones produced in that fat are a precipitating cause of insulin resistance.

- Increased blood triglyceride levels.
- Increased blood pressure; many people with hypertension are either diabetic or prediabetic and have elevated insulin levels due to insulin resistance; one of insulin's effects is to control arterial wall tension throughout the body.
- Increased pro-inflammatory cytokines associated with cardiovascular disease
- Depression due to the deranged metabolism resulting from insulin resistance (Mayfield, 1998).

2.4.4 Clinical Syndromes Associated with Insulin Resistance

The most common clinical syndromes associated with insulin resistance would be type 2 diabetes and metabolic syndrome. Others include hypertension PCOS, non-alcoholic fatty liver disease, certain forms of cancer and OSA (Reaven, 2004).

There are also relatively common conditions where insulin resistance is a secondary phenomenon; these include acute illness, hepatic cirrhosis, renal failure, pregnancy, hyperthyroidism, Cushing's disease and Cushing's syndrome as well as acromegaly and phaeochromocytoma which areless common. In many of these, the insulin resistance is due to increased production of counter-regulatory hormones (Withers and White, 2000).

2.4.5 Diagnosis of insulin resistance

Measuring the insulin resistance in isolation is inapplicable. Depending on the type and size of the research performed the choice of test or procedure used. Insulin resistance different techniques of assessment Include:

Hyperinsulinaemic euglycaemic clamp

The hyperinsulinaemic–euglycaemic glucose (HIEG) clamp test is accepted as the gold standard procedure for the assessment of insulin sensitivity in clinical research.

As defined by DeFronzo et al., the procedure consists of both a constant intravenous infusion of insulin to establish an artificially constant hyperinsulinaemic state and an infusion of variable glucose to maintain an euglycaemic state. For this method, both the glucose and insulin infusions require intravenous catheterization of one arm throughout the test.

During the test the difference in arteriovenous blood glucose increases in proportion to the rate of insulin infusion and sensitivity to insulin. Thus, adjustment of the rate of glucose infusion (GIR) from venous glycaemia will lead to overestimation of insulin sensitivity.

The procedure involves infusing glucose so that hyperglycaemia can be achieved easily. Therefore, as with the HIEG clamp the infusion of glucose is adapted to keep the level of hyperglycaemia typically at 200 mg / dL (11.0 mmol / L) (DeFronzo RA, Tobin JD, 1979).

Oral glucose tolerance test (OGTT)

The oral glucose tolerance test (OGTT) is a simple test that is commonly used for the diagnosis of glucose intolerance and type 2 diabetes in clinical settings. Whereas fasting and 2 h postload glucose values are adequate for clinical diagnosis, additional samples are collected every 30 min for both plasma insulin and glucose following an oral glucose load (75 g) can allow estimation of insulin sensitivity and/or secretion. The OGTT is less reliable than hyperinsulinemic-euglycemic clamp technique (Matsuda and DeFronzo, 1999).

Homeostatic model assessment HOMA

Matthews et al developed the first assessment of the homeostasis model in 1985. It is a method used to quantify insulin resistance and beta cell function from basal (fast) glucose and insulin (or C-peptide) concentrations. HOMA is a model of glucose-insulin dynamics relationship that predicts fast steady-state glucose and insulin concentrations for a wide range of possible combinations of insulin resistance and β-cell function. HOMA describes this glucose-insulin homeostasis by means of a set of simple, mathematically equations. The approximating equation for insulin resistance has been simplified; it uses a fasting blood sample. It is derived from the use of the insulin-glucose product, divided by a constant (Gutch *et al.*, 2015).

Insulin suppression test IST

The insulin suppression test (IST), described by Shen et al is another method that directly measures insulin sensitivity/resistance. After an overnight fast, somatostatin (250 g/h) is intravenously infused to suppress the endogenous production of insulin. At the same time, glucose (6 mg/kg body weight/min) and insulin (50 mU/min) are infused over 150 min at a constant rate. Glucose and insulin determinations are performed every 30 min for 2.5 h, then at 10 min intervals from 150 to 180 min of the IST. The resulting SSPG concentration obtained during the last 30 min of infusion represents an estimation of tissue insulin sensitivity. The higher the SSPG concentration, the more insulin-resistant the individual is. The IST was the first test to use steady-state plasma insulin levels to promote disposal of a glucose load (Shen, Reaven and Farquhar, 1970).

2.4.6 Management of insulin resistance

The main step in insulin resistance management is nutrition that includes low caloric intake to reverse overweight and obesity plus dietary carbohydrate adjustment to prevent excess insulin from getting worse. Lifestyle change, including diet modification and exercise, is considered the first line for the management of type 2 diabetes mellitus. If no progress has been made within 3 months, the drug will be the second choice (Conlon *et al.*, 2013).

Chapter Three
Materials and Methods

3. Materials and methods

3.1. Materials

3.1.1 Study design: This is an observational analytical quasi study.

3.1.2 Study area and period: The study was conducted in Sudanese obese females in Khartoum state over 3 months from September to December 2019.

3.1.3 Ethical consideration

After approving of the study from Department of Clinical Chemistry in college of Medical laboratory science in Sudan University of Science and Technology a written informed consent was obtained from each participant, data was collected using questionnaire.

3.1.4 Study populations: This study included 20 obese Sudanese females; the mean of age was 30, while BMI $\geq$ 31.

Inclusion criteria: Sudanese obese females were included.

Exclusion criteria: females who have insulinoma, using drugs that induced hyperinsulinemia or increase insulin sensitivity and women who are diabetic, hypertensive, Pregnant or have polycystic ovarian syndrome, impaired renal or liver function were excluded.

3.1.5 Sampling:

Two blood samples were obtained from the patients, first one before starting the ketogenic diet and the second one after three months of following the diet.

Fasting blood samples were collected by using dry, plastic syringes, tourniquet was used, and 5ml of blood was withdrawn, equal volume of blood was dispensed in a fluoride oxalate and plain containers. Fluoride oxalated blood separated as soon as possible by centrifugation at 4000 rpm to obtain serum.

Blood in Plain containers were allowed to clot at room temperature then they were centrifuged at 4000 rpm to obtain serum.

3.2 Methods:

3.2.1 Calculation of BMI

Body Mass Index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared.

3.2.2 Estimation of glucose

Fasting glucose level was estimated using glucose oxidase method.

Principle of the method

Glucose oxidase (GOD) catalyzes the oxidation of glucose to gluconic acid. The formed hydrogen peroxide (H2O2) is detected by a chromogenic oxygen acceptor, phenol; 4–aminophenazone (4-AP) in the presence of peroxidase (POD).The intensity of the color formed is proportional to the glucose concentration in the sample.

Procedures

Fully automated device is used (COBUS c111).

3.2.3 Insulin estimation:

Insulin levels were determined using immunometric method using Tosoh kits and apparatus.

- Principle: Immunoenzymatic assay were used. Insulin present in the test sample is bound with monoclonal antibody immobilized on a magnetic solid phase and enzyme-labeled monoclonal antibody in the test cups. The amount of enzyme-labeled monoclonal antibody that binds to the beads is directly proportional to the insulin concentration in the test sample.

Procedures

Fully automated device is used (TOSOH 1800).

3.2.4 Calculation of insulin resistance index

Insulin resistance index is calculated using HOMA equation which state that:

$$IR = \frac{\text{FBG (mmol/l) x fasting insulin (μU/ml)}}{22.5}$$

3.2.5 Diet restriction

All 20 subjects were instructed to follow a ketogenic diet consisting of less than 5% of carbohydrates and 25% of proteins and 70% of fat. metabolic rate as determined by the Harris Benedic equation, the calories consumed ranged between1600 to 2200 kcal per day. Polyunsaturated and monounsaturated fats were included in the diet. A list of recommended and restricted food in ketogenic diet is given.

Micronutrients (vitamins and minerals) in the form of one capsule/day were recommended to each subject throughout the study period.

3.3 Quality control:

Normal and pathological control sera were included within every batch of chemical analysis to insure the accuracy of the results.

3.4 Statistical analysis:

Paired-samples T test was used to compare mean of concentrations of the study parameters, ANOVA test for repeated measures was used to measure the differences between the means of weight at the different points of time, Person correlation test is used to find the correlation between age and Insulin resistance before and after ketogenic diet.

All analysis was analyzed by using the (SPSS) computer program.

Significant change considered as P.value > 0.05.

Chapter Four
Results

4. Results

In this study, twenty obese Sudanese females served as study group their age ranged from 22 to 39 they were included in this study to assess the impact of ketogenic diet on insulin resistance index, Only 15 participants (75%) completed 12 weeks successfully. data were analyzed statistically using computer SPSS program and the result were as follow.

Table 4.1 shows a significant reduction in fasting blood glucose level when compared to the level before and after the diet (mean 87.8±6.4, 80.2±7.2 mg/dl, P.value= 0.00 respectively).

It shows a significant decrease in fasting insulin level when compared to the level before and after the diet (mean 11.5±6, 7.4±4.8 μU/ml, P.value= 0.00 respectively).

In addition, there is a significant decrease in insulin resistance when compared to the level before and after the diet (mean 2.5±1.3, 1.5±0.9, P.value= 0.00 respectively).

Also, it shows a significant decrease in the weight when compared to the weight before and after the diet (mean 101.3±10.3, 90.3±8.6 Kg, P. value= 0.00 respectively).and significant reduction in BMI when compared to the BMI before and after the diet (mean 36.5±3.9, 32.5±3.2 Kg/M^2, P. value= 0.00 respectively).

Table 4.2: The Sphericity test shows that the differences of weight between all combinations of related groups is not equal, (P.value = 0.00).

Table 4.3: shows there is a statistically significant difference between the means of weight at the different points of time, (P.value= 0.00).

Table 4.4: Pairwise Comparisons shows a significant difference in weight loss between all weeks (P.value is < 0.005) except for week 4 and week 6, there is no significant differences (P.value = 0.095).

Table 4.5: shows no statistically significant correlation between age and Insulin resistance before and after the ketogenic diet, (P-value = 0.804, 0.605, respectively).

Figure 4.1 shows the differences between the means of weight at the different points of time.

Table 4.1: Comparison between means of fasting blood glucose, fasting insulin, insulin resistance, (weight and BMI) in Sudanese females before and after the diet.

Variable	before the diet (Mean±SD)	after the diet (Mean±SD)	P.value
Fasting blood glucose (mg/dl)	87.8±6.4	80.2±7.2	.000
Fasting insulin (μU/ml)	11.5±6	7.4±4.8	.000
Insulin resistance	2.5±1.3	1.5±0.9	.000
Weight (kg)	101.3±10.3	90.3±8.6	.000
BMI (Kg/m^2)	36.5±3.9	32.5±3.2	.000

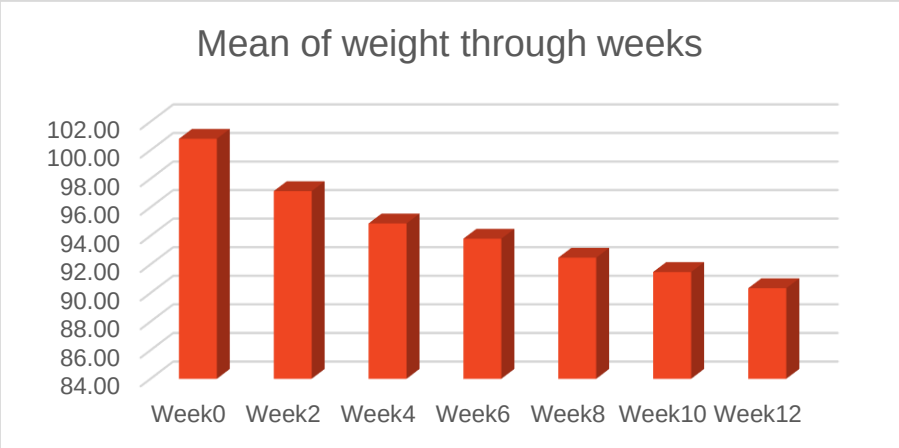

Figure 4.1: The differences between the means of weight at the different points of time.

Table 4.2: Results of ANOVA for repeated measures: Mauchly's Test of Sphericity

Mauchly's Test of Sphericity[a]							
Measure: Weight							
Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Time	.003	68.299	20	.000	.346	.408	.167

Each point of time represent a group, P-value is < 0.05 reject the null hypothesis (the differences of weight between all combinations of related groups is equal), therefore Sphericity is violated, the Greenhouse-Geisser correction is needed.

Table 4.3: Results of ANOVA for repeated measures: Tests of Within-Subjects Effects table.

Tests of Within-Subjects Effects							
Measure: Weight							
Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Time	Sphericity Assumed	1168.324	6	194.721	110.333	.000	.887
	Greenhouse-Geisser	1168.324	2.078	562.155	110.333	.000	.887
	Huynh-Feldt	1168.324	2.447	477.419	110.333	.000	.887
	Lower-bound	1168.324	1.000	1168.324	110.333	.000	.887
Error(Time)	Sphericity Assumed	148.248	84	1.765			
	Greenhouse-Geisser	148.248	29.096	5.095			
	Huynh-Feldt	148.248	34.260	4.327			
	Lower-bound	148.248	14.000	10.589			

"**Greenhouse-Geisser**" (F (2.078, 29.096) = 110.333, $p=0.000 < 0.05$)

Table 4.4: Pairwise Comparisons results

Pairwise Comparisons						
Measure: Weight						
(I) Time		**Mean Difference (I-J)**	**Std. Error**	**Sig.**	**95% Confidence Interval for Difference**	
					Lower Bound	Upper Bound
0	2	3.667*	0.566	0.000	1.572	5.761
	4	5.933*	0.613	0.000	3.665	8.201
	6	7.000*	0.617	0.000	4.717	9.283
	8	8.333*	0.695	0.000	5.764	10.903
	10	9.333*	0.797	0.000	6.386	12.281
	12	10.467*	0.786	0.000	7.559	13.374
2	0	-3.667-*	0.566	0.000	-5.761	-1.572
	4	2.267*	0.206	0.000	1.504	3.03
	6	3.333*	0.422	0.000	1.774	4.893
	8	4.667*	0.454	0.000	2.986	6.347
	10	5.667*	0.566	0.000	3.572	7.761
	12	6.800*	0.579	0.000	4.658	8.942
4	0	-5.933-*	0.613	0.000	-8.201	-3.665
	2	-2.267-*	0.206	0.000	-3.03	-1.504
	6	1.067	0.316	0.095	-0.101	2.235
	8	2.400*	0.335	0.000	1.16	3.64
	10	3.400*	0.445	0.000	1.754	5.046
	12	4.533*	0.456	0.000	2.845	6.221
6	0	-7.000-*	0.617	0.000	-9.283	-4.717
	2	-3.333-*	0.422	0.000	-4.893	-1.774
	4	-1.067	0.316	0.095	-2.235	0.101
	8	1.333*	0.211	0.000	0.553	2.113
	10	2.333*	0.347	0.000	1.048	3.618
	12	3.467*	0.336	0.000	2.223	4.71
8	0	-8.333-*	0.695	0.000	-10.903	-5.764
	2	-4.667-*	0.454	0.000	-6.347	-2.986
	4	-2.400-*	0.335	0.000	-3.64	-1.16
	6	-1.333-*	0.211	0.000	-2.113	-0.553
	10	1.000*	0.218	0.009	0.193	1.807
	12	2.133*	0.236	0.000	1.259	3.008
10	0	-9.333-*	0.797	0.000	-12.281	-6.386
	2	-5.667-*	0.566	0.000	-7.761	-3.572
	4	-3.400-*	0.445	0.000	-5.046	-1.754
	6	-2.333-*	0.347	0.000	-3.618	-1.048
	8	-1.000-*	0.218	0.009	-1.807	-0.193
	12	1.133*	0.215	0.003	0.337	1.93
12	0	-10.467-*	0.786	0.000	-13.374	-7.559
	2	-6.800-*	0.579	0.000	-8.942	-4.658
	4	-4.533-*	0.456	0.000	-6.221	-2.845
	6	-3.467-*	0.336	0.000	-4.71	-2.223

	8	-2.133-*	0.236	0.000	-3.008	-1.259
	10	-1.133-*	0.215	0.003	-1.93	-0.337

The "**Mean Difference (I-J)**" column shows how much the weight loss from one week to the other E.g. the mean of week_0 weight is 3.667 times higher than week_2.

Table 4.5: The correlation between age and Insulin resistance before and after ketogenic diet

		Pre IR	**Post IR**
Age	Pearson Correlation	-0.07	-0.145
	Sig. (2-tailed)	0.804	0.605

Insulin resistance before and after the diet (P-value > 0.05)

Chapter Five
Discussion, Conclusion and Recommendations

5. Discussion, conclusion and recommendations

5.1 Discussion:

Obesity has a large impact on insulin resistance and contributes substantially and directly to cardiovascular risk as well as exacerbating associated risk factors such as dyslipidaemia, hypertension and diabetes (Noakes *et al.*, 2006).

Among various diets, ketogenic diet, which are very low in carbohydrates and usually high in fats and proteins, have gained in popularity (Kosinski and Jornayvaz, 2017).

This quasi study aimed to study the impact of ketogenic diet on insulin resistance index, 15 obese Sudanese females were enrolled in this study. After evaluation of insulin resistance before and after the diet in duration of three months, the result showed that the insulin resistance was significantly decresed.This result is in agreed with study done by (Samaha *et al.*, 2003) and (Johnstone *et al.*, 2008).

in fact, due to carbohydrate restriction glucose availability will reduced and lower insulin output, this may increase the concentration of counter regulatory hormones such as catecholamines and glucagon, thereby promoting adipose tissue lipolysis.Since the insulin resistance hinders the ability to take up plasma glucose, which results in glucose diversion to the liver where it is converted to and stored as fat. In contrast, nutritional ketosis reduces insulin levels thereby suppressing lipogenesis.

Another study support this result done by (Foster *et al.*, 2003),which described a better insulin sensitivity by consuming a KD, but this improvement does not seem to be permanent that is possibly due to the enhanced effect of carbohydrate-restriction on glycemic control is most significant during the first 3 to 6 months and becomes less effective thereafter (Westman *et al.*, 2018).

In contrast, a study conducted by (Bisschop *et al.*, 2001) showed that a high-fat, low-carbohydrate ketogenic diet reduces the ability of insulin to suppress endogenous glucose production in healthy men. The limitation that it studied the impact of different amounts of dietary fat on insulin in short period (only 11 days), which is possibly not sufficient for body to adapt to ketone utilization.

The current study results revealed that there is decrease in fasting blood glucose level before and after the ketogenic diet. this result agreed with study done by (Johnstone *et al.*, 2008), this is due to reduction in the availability of dietary glucose; progressively less insulin was secreted to maintain the same blood glucose concentrations.

This finding disagree with studies done by (Jabekk *et al.*, 2010) and (Sun *et al.*, 2019), which showed no significant difference on blood glucose level before and after the diet. the researchers attributed that to differences in Low Carbohydrate dietary approaches.

In the present study there was significant decrease in weight and BMI, The Sphericity test shows that the differences of weight between all combinations of related groups is not equal, concluding that there is a statistically significant difference between the means of weight at the different Points of time. This finding agreed with studies done by (Johnstone *et al.*, 2008) and (Meckling *et al.*, 2002).

This significant reduction during first weeks is possibly due to ketogenic diet effect of accelerate weight loss by inducing sodium and water deficits and reduced caloric intake, another hypothesis of KD-induced weight loss is decreased appetite induced by ketosis.

The current results didn't give proof on direct correlation between insulin resistance and age before and after the ketogenic diet, previous study demonstrate that age-related reductions in insulin sensitivity are likely due to an age-related increase in adiposity rather than a consequence of advanced chronological (Karakelides *et al.*, 2010).

5.2 Conclusion

There is decrease of insulin resistance, weight and BMI among Sudanese obese females after following the ketogenic diet for three months. Therefore ketogenic diet has a positive impact on body weight and insulin resistance index .

5.3 Recommendations

From the result of this study it is recommended that:

- Ketogenic diet is recommended dietary therapy to lose weight and reduce insulin resistance.
- Obese individuals recommnded to consult a physican before starting the ketogenic diet.
- During the ketogenic diet, a daily variety of foods is recommnded to avoid nutrient deficiencies.
- Insulin level, as well as lipid profile, glucose and hemostatic indices recommended to be measured monthly for obese people to monitor the effects of obesity and prevent the complications.
- Further studies with long term period recommended to be done to confirm the positive effect of ketogenic diet.

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Appendices

استمارة موافقة الشخص المشارك في البحث

أنا الباحث: الشيماء قسم الله محمد، كلية الدراسات العليا، جامعة السودان للعلوم والتكنولوجيا، أقوم ببحث ودراسة تأثير حمية الكيتوجينيك على مقاومة الأنسولين لدى النساء بولاية الخرطوم.

The impact of ketogenic diet on insulin resistance in Sudanese females in Khartoum State.

لقد تم اختيارك لتشارك في هذا البحث ومعك عدد آخر من المشاركين.

نتوقع بمشاركتك أنت والمشاركين الآخرين أن نتحصل على نتائج تفيد فهم مدى تأثير حمية الكيتوجينيك على مقاومة الأنسولين، وقد يعود البحث بفوائد على كل من المشارك نفسه أو المجتمع أو مقدمي الخدمات الصحية.

خلال هذه الدراسة سأقوم بأخذ معلومات عنك وأخذ عينة دم من الوريد حوالي 5ملليتر، ثم إجراء قياس السكر في حالة الصيام، وقياس مستوى هرمون الأنسولين من عينة الدم.

الإجراء الذي سأقوم به تجاهك ليس به أية مخاطر أو أعراض جانبية على المشارك.

ونحن إذ نأمل في مشاركتك معنا في هذا البحث، نؤكد لك على سرية المعلومات والوثائق الخاصة بك، وأنه لن يطلع عليها إلا الباحث المعني.

بعد اطلاعك على نتيجة الفحوصات سنقوم بمدَك بالتعليمات اللازمة لإتباع الحمية بطريقة صحيحة، سيستمر البحث ثلاثة أشهر تستوجب الالتزام بالنظام ابتداء من 1 سبتمبر حتى 1 ديسمبر، سنقوم بعدها بجمع عينة أخرى منك.

سأقوم بأخذ معلومات منك تتعلق ب

الوزن:	الطول:	العمر:
BMI :	الحالة الاجتماعية:	رقم الهاتف:

العينات التي يتم جمعها سوف تستعمل لغرض هذه الدراسة فقط. ونود أن نشير كذلك إلى أن المشاركة في البحث طوعية وأن رفضك للمشاركة في البحث لا تفقدك الحق في أي فوائد من البحث، مع التأكيد على أنه لن يتم منحك أي قيمة نقدية مقابل المشاركة في هذا البحث وأنه بمشاركتك ستكون أحد المتطوعين الذين يشملهم البحث.

إذا كان لديك أي سؤال أو استفسار يخص البحث يمكنك التواصل مع الباحث أعلاه في رقم جواله:

إقرار موافقة المشارك في البحث

إقرار المشارك:
لقد اطلعت على المعلومات الحالية والتي تم شرحها لي وأتيح لي طرح الأسئلة عنها كيفما شئت، وقد تلقيت الإجابات الوافية عن كل الأسئلة، وأنا أقر بالموافقة على المشاركة طواعية في هذه الدراسة وأعلم بحقي في التوقف عن المشاركة في أي وقت دون أن يؤثر ذلك عليَ.
رمز المشارك: .....
اسم المشارك: .....
توقيع المشارك: .....

اسم الباحث:.....

توقيع الباحث:.....

Questionnaire

Sudan University of Science and Technology

The Impact of Ketogenic Diet on Body Weight and Insulin Resistance Index among Sudanese obese females

Name:…….……
No:……...….....
Age:…….
Height/cm:…….....
Weight/ Kg……...
BMI: ……

Are you pregenant?

Yes ☐ NO ☐

Did you have any health problems?

Diabetes Yes ☐ NO ☐

Hypertension Yes ☐ NO ☐

Polycystic ovarian syndrome Yes ☐ NO ☐

Renal diseases Yes ☐ NO ☐

Liver diseases Yes ☐ NO ☐

Cardiac diseases Yes ☐ NO ☐

Have you ever tried high fat low carbohydarte diet?
Yes ☐ NO ☐

04773655001V11.0

GLUC2

Glucose HK

cobas®

Urine
Collect urine in a dark bottle. For 24 hours urine collections, glucose may be preserved by adding 5 mL of glacial acetic acid to the container before collection. Unpreserved urine samples may lose up to 40 % of their glucose after 24-hours storage at room temperature.[3] Therefore, keep samples on ice during collection.[5]

Centrifuge samples containing precipitates before performing the assay.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

See "Order information" section

General laboratory equipment

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum, plasma and urine

cobas c 111 test definition

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction direction	Increase
Wavelength A/B	340/409 nm
Calc. first/last (serum, plasma)	16/37
Calc. first/last (urine)	16/38
Unit	mmol/L
Reaction mode	R1-S-SR

Pipetting parameters

		Diluent (H_2O)
R1	150 µL	
Sample	2 µL	20 µL
SR	30 µL	
Total volume	202 µL	

Calibration

Calibrators	Calibrator f.a.s.
	Deionized water is used automatically by the instrument as the zero calibrator.
Calibration mode	Linear regression
Calibration interval	Each lot and as required following quality control procedures.

Traceability: This method has been standardized against ID/MS.

Quality control

Serum/plasma

For quality control, use control materials as listed in the "Order information" section. In addition, other suitable control material can be used.

Urine

Quantitative urine controls are recommended for routine quality control.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The **cobas c** 111 analyzer automatically calculates the analyte concentration of each sample.

Conversion factors:	mmol/L × 18.02 = mg/dL
	mmol/L × 0.1802 = g/L
	mg/dL × 0.0555 = mmol/L

Limitations - interference

Criterion: Recovery within ± 10 % of initial value at a glucose concentration of 6.38 mmol/L (115 mg/dL).

Serum/plasma

Icterus:[7] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:[7] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):[7] No significant interference up to an L index of 2000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[8,9]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[10]

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

NOTE: Glucose values achieved on some proficiency testing materials, when evaluated against a glucose oxidase-oxygen electrode comparison method, demonstrate an approximate 3 % positive bias on average.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on the **cobas c** 111 analyzer. For information about test combinations requiring special wash steps, please refer to the latest version of the carry over evasion list found with the CLEAN Method Sheet and the operator's manual for further instructions.

Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range

Serum, plasma and urine:
0.11-40 mmol/L (1.98-720 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:10 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 10.

Lower limits of measurement

Lower detection limit of the test:
0.11 mmol/L (1.98 mg/dL)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying 3 standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

Expected values

Plasma[11]

Fasting	4.11-6.05 mmol/L	(74-109 mg/dL)

Urine[12]

1st morning urine	0.3-1.1 mmol/L	(6-20 mg/dL)
24 hour urine	0.3-0.96 mmol/L	(6-17 mg/dL)
	(average of 1350 mL urine/24 h)	

GLUC2

Glucose HK

cobas®

acc. to Tietz[5]

Serum, plasma

Adults	4.11-5.89 mmol/L	(74-106 mg/dL)
60-90 years	4.56-6.38 mmol/L	(82-115 mg/dL)
> 90 years	4.16-6.72 mmol/L	(75-121 mg/dL)
Children	3.33-5.55 mmol/L	(60-100 mg/dL)
Neonates (1 day)	2.22-3.33 mmol/L	(40-60 mg/dL)
Neonates (> 1 day)	2.78-4.44 mmol/L	(50-80 mg/dL)

Urine

24 hour urine	< 2.78 mmol/24 h	(< 0.5 g/24 h)
Random urine	0.06-0.83 mmol/L	(1-15 mg/dL)

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the **cobas c** 111 analyzer are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in an internal protocol with repeatability (n = 21) and intermediate precision (3 aliquots per run, 1 run per day, 10 days). The following results were obtained:

Serum, plasma

Repeatability	*Mean mmol/L (mg/dL)*	*SD mmol/L (mg/dL)*	*CV %*
Precinorm U	5.03 (90.6)	0.05 (0.9)	1.0
Precipath U	14.0 (252)	0.1 (2)	0.5
Human serum 1	2.27 (40.9)	0.03 (0.5)	1.1
Human serum 2	10.0 (180)	0.1 (2)	0.8

Serum, plasma

Intermediate precision	*Mean mmol/L (mg/dL)*	*SD mmol/L (mg/dL)*	*CV %*
Precinorm U	5.12 (92.3)	0.03 (0.5)	0.7
Precipath U	14.1 (254)	0.1 (2)	0.5
Human serum 1	2.52 (45.4)	0.01 (0.2)	0.5
Human serum 2	9.89 (178)	0.06 (1)	0.6

Urine

Repeatability	*Mean mmol/L (mg/dL)*	*SD mmol/L (mg/dL)*	*CV %*
Control level 1	1.90 (34.2)	0.01 (0.18)	0.7
Control level 2	15.7 (283)	0.04 (0.72)	0.3
Urine sample 1	0.80 (14.4)	0.01 (0.18)	1.6
Urine sample 2	30.0 (541)	0.10 (1.80)	0.4

Method comparison

Glucose values for human serum, plasma and urine samples obtained on a **cobas c** 111 analyzer (y) were compared with those determined using the corresponding reagent on a COBAS INTEGRA 400 analyzer (x).

Serum/plasma

Sample size (n) = 80

Passing/Bablok[13]	Linear regression
y = 1.022x – 0.009 mmol/L	y = 1.021x + 0.019 mmol/L
τ = 0.983	r = 1.000

The sample concentrations were between 2.2 and 29.8 mmol/L (39.6 and 537 mg/dL).

Urine

Sample size (n) = 54

Passing/Bablok[13]	Linear regression
y = 0.984x – 0.007 mmol/L	y = 0.986x – 0.047 mmol/L
τ = 0.991	r = 1.000

The sample concentrations were between 0.127 and 39.1 mmol/L (2.34 and 705 mg/dL).

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A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard:

CONTENT	Contents of kit
REAGENT	Reagent
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

04772065001V11.0

GLUC2

Glucose HK

cobas®

Order information

REF	CONTENT	Analyzer(s) on which kit(s) can be used
		cobas c 111
04657527 190	Glucose HK (4 x 100 tests)	Code 401
10759350 190	Calibrator f.a.s. (12 x 3 mL)	Code 300
12149435 122	Precinorm U plus (10 x 3 mL)	Code 301
12149443 122	Precipath U plus (10 x 3 mL)	Code 300
10171743 122	Precinorm U (20 x 5 mL)	Code 300
10171735 122	Precinorm U (4 x 5 mL)	Code 301
10171778 122	Precipath U (20 x 5 mL)	Code 301
10171760 122	Precipath U (4 x 5 mL)	Code 391
05117003 190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 391
05947626 190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 392
05117216 190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 392
05947774 190	PreciControl ClinChem Multi 2 (4 x 5 mL)	

English

System information

GLU2: ACN 767

GLU2U: ACN 305

Applications available on request:

GLUH2: ACN 409 (hemolysate)

GLU2P: ACN 756 (hemolysate plasma-level)

A separate method sheet for the hemolysate applications is available on request.

Intended use

In vitro test for the quantitative determination of glucose in human serum, plasma and urine on the ***cobas c*** *111 system.*

Summary[1,2,3]

Glucose is the major carbohydrate present in the peripheral blood. Oxidation of glucose is the major source of cellular energy in the body. Glucose derived from dietary sources is converted to glycogen for storage in the liver or to fatty acids for storage in adipose tissue. The concentration of glucose in blood is controlled within narrow limits by many hormones, the most important of which are produced by the pancreas.

The most frequent cause of hyperglycemia is diabetes mellitus resulting from a deficiency in insulin secretion or action. A number of secondary factors also contribute to elevated blood glucose levels. These include pancreatitis, thyroid dysfunction, renal failure and liver disease.

Hypoglycemia is less frequently observed. A variety of conditions may cause low blood glucose levels such as insulinoma, hypopituitarism or insulin induced hypoglycemia.

Glucose measurement in urine is used as a diabetes screening procedure and to aid in the evaluation of glucosuria, to detect renal tubular defects, and in the management of diabetes mellitus.

Test principle

UV test

Enzymatic reference method with hexokinase.[4,5]

Hexokinase catalyzes the phosphorylation of glucose to glucose-6-phosphate by ATP.

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{G-6-P} + \text{ADP}$$

Glucose-6-phosphate dehydrogenase oxidizes glucose-6-phosphate in the presence of NADP to gluconate-6-phosphate. No other carbohydrate is oxidized. The rate of NADPH formation during the reaction is directly proportional to the glucose concentration and is measured photometrically.

$$\text{G-6-P} + \text{NADP}^+ \xrightarrow{\text{G-6-PDH}} \text{gluconate-6-P} + \text{NADPH} + \text{H}^+$$

Reagents - working solutions

R1 TRIS buffer: 100 mmol/L, pH 7.8; Mg^{2+}: 4 mmol/L; ATP: ≥ 1.7 mmol/L; NADP: ≥ 1.0 mmol/L; preservative

SR HEPES buffer: 30 mmol/L, pH 7.0; Mg^{2+}: 4 mmol/L; HK (yeast): ≥ 130 µkat/L; G-6-PDH (*E. coli*): ≥ 250 µkat/L; preservative

Precautions and warnings

For *in vitro* diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

Reagent handling

Ready for use

Storage and stability

Shelf life at 2-8 °C:	See expiration date on reagent
On-board in use and refrigerated on the analyzer:	4 weeks

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable:
Serum
Plasma: Li-heparin, K_3-EDTA, NaF/Na_2-EDTA, NaF/citrate/Na_2-EDTA, KF/Na_2-EDTA or NaF/K-oxalate plasma.

The stability of glucose in specimens is affected by storage temperature, bacterial contamination, and glycolysis. Plasma or serum samples without preservative (NaF) should be separated from the cells or clot within half an hour of being drawn. When blood is drawn and permitted to clot and to stand uncentrifuged at room temperature, the average decrease in serum glucose is ~ 7 % in 1 hour (0.28-0.56 mmol/L or 5-10 mg/dL). This decrease is the result of glycolysis. Glycolysis can be inhibited by collecting the specimen in fluoride tubes.[1]

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Stability (no hemolysis):[5]	8 hours at 15-25 °C
	72 hours at 2-8 °C
Stability in Na-fluoride plasma:[6]	3 days at 20-25 °C

ST AIA-PACK IRI

For Quantitative Measurement of insulin (IRI) in Serum, Heparinized Plasma or EDTA Plasma

NAME AND INTENDED USE

ST AIA-PACK IRI is designed for IN VITRO DIAGNOSTIC USE ONLY for the quantitative measurement of insulin (IRI) in human serum, heparinized plasma or EDTA plasma on TOSOH AIA System Analyzers.

SUMMARY AND EXPLANATION OF TEST

Insulin, the antidiabetic hormone, is produced in the pancreatic B cell as a large preproinsulin containing 109 amino acid residues with a molecular weight of approximately 11,500 *(1, 2)*. This peptide is rapidly converted by cleavage to proinsulin consisting of 86 amino acid residues with a molecular weight of approximately 9,000 and is stored within the B cell secretory granules *(3)*. Equimolar quantities of insulin (51 amino acids with a molecular weight of approximately 6,000) and C-peptide (31 amino acids, molecular weight of approximately 3,000) are produced through proteolytic cleavage and then secreted along with a small amount of proinsulin *(4)*. Insulin exists in polymeric forms depending on the pH and zinc content. The monometric insulin molecule is composed of two polypeptide chains, "alpha" and "beta" which are connected by two interchain disulfide bridges of cystine *(3)*.

Insulin is degraded in most tissues and has a plasma half-life of 7 - 15 minutes in man. It is also rapidly and completely inactivated in the gastrointestinal tract. Daily production of insulin in a healthy adult is 40 - 50 units. After binding to its specific receptors on target cell membranes, insulin acts as an anabolic and anticatabolic hormone, influencing the rates of carbohydrate, lipid, protein and electrolyte metabolism *(5-7)*.

Insulin release is stimulated by glucose. A failure to respond to this glucose stimulus may be one of the fundamental defects in human diabetes *(8-10)*. The factors stimulating or inhibiting insulin release or the factors decreasing the tissue response to insulin have been well documented.

PRINCIPLE OF THE ASSAY

The ST AIA-PACK IRI is a two-site immunoenzymometric assay which is performed entire in the ST AIA-PACK IRI test cups. Insulin present in the test sample is bound with monoclor antibody immobilized on a magnetic solid phase and enzyme-labeled monoclonal antibody the test cups. The magnetic beads are washed to remove unbound enzyme-labeled monoclor antibody and are then incubated with a fluorogenic substrate, 4-methylumbelliferyl phospha (4MUP). The amount of enzyme-labeled monoclonal antibody that binds to the beads is direct proportional to the insulin concentration in the test sample. A standard curve is constructed, a unknown sample concentrations are calculated using this curve.

MATERIAL PROVIDED (ST AIA-PACK IRI, Cat. No. 0025260)

5 trays x 20 test cups

Plastic test cups containing lyophilized eight magnetic beads coated with anti-insulin mou monoclonal antibody and 100 μL of anti-insulin mouse monoclonal antibody conjugated to bovir alkaline phosphatase with sodium azide as a preservative.

WARNINGS AND PRECAUTIONS

1. The ST AIA-PACK IRI is intended for in vitro diagnostic use only.
2. Inspect the packaging and the exterior of the aluminum pouch for any sign of dam use. If any damages are visible, contact your local TOSOH sales representative.
3. Test cups from different lots or different assays shall not be mixed within a tray.
4. The ST AIA-PACK IRI contains sodium azide, which may react with lead or copper to form potentially explosive metal azides. When disposing of such reagents, al with large volumes of water to prevent azide build-up.
5. Human serum is not used in the preparation of this product; however, since human will be used for samples and other quality control products in the lab may be de human serum, please use standard laboratory safety procedures in handling all spe controls.
6. Do not use beyond the expiration date.
7. The ST AIA-PACK IRI has been designed so that the high dose "hook effect" is no for the vast majority of samples. Samples with insulin concentrations betwee 20,000 μU/mL will read > 320 μU/mL. The "hook effect" phenomenon may occu concentrations > 20,000 μU/mL.
8. For safe waste disposal, it is recommended that each laboratory complies with laboratory procedures and local, state, and federal regulations.
9. After opening, the vial of AIA-PACK IRI SAMPLE DILUTING SOLUTION sho tightly sealed with a clean rubber cap. Sealing with dirty material may cause dete the reagent.
10. The remaining sample diluting solution after use should not be mixed with another discarded to avoid contamination.
11. Serum, dust, metal, or microorganism contamination may cause degradation of re substrate solution. Store in a clean environment, away from direct sunlight and light.
12. TOSOH recommends that a new pouch of the test cups should be used for calibrat

STORAGE AND STABILITY

All unopened materials are stable until the expiration date on the label when stored at t temperature.

Materials	Cat. No.
2-8 °C:	
ST AIA-PACK IRI	0025260
AIA-PACK IRI CALIBRATOR SET	0020360
AIA-PACK IRI SAMPLE DILUTING SOLUTION	0020560
AIA-PACK SUBSTRATE SET II	0020968
AIA-PACK WASH CONCENTRATE	0020955
AIA-PACK DILUENT CONCENTRATE	0020956
1-30 °C:	
AIA-PACK DETECTOR STANDARDIZATION TEST CUP	0020970
AIA-PACK SAMPLE TREATMENT CUP	0020971

After opening the aluminum pouch, ST AIA-PACK IRI test cups can be left on-b TOSOH AIA System Analyzers (18-25 °C) for a maximum of 4 days (4 x 24 hours). over night at 2-8 °C, the test cups can be used for up to 12 days (12 cycles of 8 hours 16 hours in the refrigerator). Once the aluminum pouch is opened, even if the test cup within 30 days. and refrigerated at 2-

PROCEDURE

For the AIA Nex·IA / AIA-21, AIA-600 II, AIA-900, AIA-1800, AIA-2000 and AIA-360, pleas refer to their Operator's Manual for detailed instructions.

I. Reagent Preparation

A) Substrate Solution

Bring all reagents to 18-25 °C before preparing the working reagent. Add the entire content: of the AIA-PACK SUBSTRATE RECONSTITUENT II (100 mL) to the lyophilized AIA-PACK SUBSTRATE REAGENT II and mix thoroughly to dissolve the solid material.

B) Wash Solution

Add the entire contents of the AIA-PACK WASH CONCENTRATE (100 mL) to approximately 2.0 L of CAP Class I water or the clinical laboratory reagent water (formerly NCCLS Type I) defined by CLSI GP40-A4-AMD guideline, mix well, and adjust the final volume to 2.5 L.

C) Diluent

Add the entire contents of the AIA-PACK DILUENT CONCENTRATE (100 mL) to approximately 4.0 L of CAP Class I water or the clinical laboratory reagent water (formerly NCCLS Type I) defined by CLSI GP40-A4-AMD guideline, mix well, and adjust the final volume to 5.0 L.

II. Calibration Procedure

A) Calibration Curve

The calibrators for use with the ST AIA-PACK IRI have been standardized on WHO 1st IRP 66/304 (1974).

The calibration curve for ST AIA-PACK IRI is stable for up to 90 days. Calibration stability is monitored by quality control performance and is dependent on proper reagent handling and TOSOH AIA System maintenance according to the manufacturer's instructions.

Recalibration may be necessary more frequently if controls are out of the established range for this assay or when certain service procedures are performed (e.g. temperature adjustment, sampling mechanism changes, maintenance of the wash probe, or detector lamp adjustment or change). For further information regarding instrument operation, consult the TOSOH AIA System Operator's Manual.