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THE ROLE OF COPAPTIN IN CHANGES SECONDARY HYPOGONADISM IN MEN WITH AND WITHOUT TYPE 1 AND 2 DM

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ASRAA FAWZI MADHI DAHAM

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By Asraa Fawzi Madhi DAHAM

January 2022

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

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Asraa Fawzi Madhi DAHAM

ABSTRACT

THE ROLE OF COPAPTIN IN CHANGES SECONDARY HYPOGONADISM IN MEN WITH AND WITHOUT TYPE 1 AND 2 DM

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This investigation is conducted to study the role of copeptin peptide in changes of the secondary hypogonadism in men with and without type1 and type of diabetes mellitus and study the correlation between copeptin and some of the biochemical parameters in men with and without Type 1and 2 diabetes mellitus. The results showed that the mean of the copeptin concentration in a blood sample of the three tested groups varied in their levels of copeptin. The results pointed out that the mean of HbA1C levels in the serum of DM2 and DM1 patients were significantly higher than in healthy persons, which recorded 9.30 and 8.06 % as compared to the control group which recorded 5.33 % respectively, the elevated in HbA1C level was associated with increases in copeptin levels considers as a biomarker of diabetes mellitus disease. We found that the elevated copeptin level is associated with the increase in lipid profile such as total triglycerides, total cholesterol HDL and LDL in diabetes patients. The results revealed that the mean of CRP levels in serum of DM2 and DM1 patients were significantly higher than in healthy persons, which recorded 12.34 pg/mL and 10.44 pg/mL as compared to control group which recorded 4.72 pg/mL respectively. The copeptin is considers as a biomarker seems to reflect the disease severity in diabetes patients.

2022, 59 pages

Keywords: T1DM, T2DM, Insulin, Kopaptin, Hipogonadizm

ÖZET

TİP 1 VE TİP 2 DM'Lİ VE TİPSİZ ERKEKLERDE SEKONDER HİPOGONADİZM DEĞİŞİKLİKLERİNDE COPAPTİN'İN ROLÜ

Asraa Fawzi Madhi DAHAM
Kimya, Yüksek Lisans
Tez Danışmanı: Prof. Dr. Volkan EYÜPOĞLU
Kasım 2022

Bu araştırma, tip1 diyabeti olan ve olmayan erkeklerde sekonder hipogonadizmdeki değişikliklerde kopeptin peptidin rolünü ve tip 1 ve tip 2 diyabeti olan ve olmayan erkeklerde kopeptin ile bazı biyokimyasal parametreler arasındaki ilişkiyi araştırmak için yapılmıştır. Sonuçlar, test edilen üç grubun bir kan örneğindeki kopeptin konsantrasyonunun ortalamasının, kopeptin seviyelerinde değişiklik gösterdiğini gösterdi. Sonuçlar, DM2 ve DM1 hastalarının serumundaki ortalama HbA1C düzeylerinin, sırasıyla %5,33 kaydeden kontrol grubuna kıyasla %9,30 ve %8,06 kaydeden sağlıklı kişilerden önemli ölçüde daha yüksek olduğuna işaret etti. Copeptin seviyelerindeki artışlarla ilişkili olarak, diabetes mellitus hastalığının bir biyobelirteç olarak kabul edilir. Yüksek kopeptin düzeyinin diyabet hastalarında toplam trigliseritler, toplam kolesterol HDL ve LDL gibi lipid profilindeki artışla ilişkili olduğunu bulduk. Sonuçlar, DM2 ve DM1 hastalarının serumundaki ortalama CRP düzeylerinin, sırasıyla 4.72 pg/mL kaydeden kontrol grubuna kıyasla 12,34 pg/mL ve 10.44 pg/mL kaydeden sağlıklı kişilerden anlamlı derecede yüksek olduğunu ortaya koydu. Bir biyobelirteç olarak kabul edilen kopeptin, diyabet hastalarında hastalık şiddetini yansıtıyor gibi görünmektedir.

2022, 59 sayfa

Anahtar Kelimeler: Apelin, Böbrek yetmezliği, Antioksidanlar, ROS

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Asraa Fawzi Madhi DAHAM
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CONTENTS

LIST OF SYMBOLS

-	Minus
%	Percent
**	Significant
/	Divide
+	Plus
<	Greater than
=	Equal
>	Less than
±	Plus minus
≤	Greater or equal to
≥	Less or equal to
Ca	Calcium
Cl	Chloride
CO_2	Carbon dioxide
Cu	Copper
dL	Deciliter
g	Gram
H	Hydrogen
HCO_3	Bicarbonate
kg	Kilogram
L	Liter
m^2	Square meter
µg	Microgram
mLU	Milli-international units
min	Minute
mL	Milliliter
mmol	Milli mole
mol	Mole
ng	Nanogram
nm	Nanometer
NS	Non-significant
P	Phosphorus
rpm	Revolutions per minute
Se	Selenium
Zn	Zinc
µL	Microliter

LIST OF ABBREVIATIONS

AMI	Acute myocardial infarction
AVP	Arginine vasopressin
BMI	Body mass index
CA	Coronary artery
CAD	Coronary artery disease
CVDs	Cardiovascular diseases
DHT	Dihydrotestosterone
DM	Diabetes mellitus
FAS	Functional assay sensitivity
FPG	Fasting plasma glucose
FSH	Follicle-stimulating hormone
GnRH	Gonadotropin-releasing hormone
hCG	Human chorionic gonadotropin
HH	Hypogonadotropic hypogonadism
IQR	Interquartile range
LH	Luteinizing hormone
LHO	Low-oestrogen hormone
MI	Myocardial infarction
SHBG	Sex hormone-binding globulin
T1DM	Type 1 Diabetes mellitus
T2DM	Type 2 Diabetes mellitus

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1. INTRODUCTION

In patients with type 2 diabetes (T2DM), abnormally high free testosterone values were discovered for the first time in 2004. These abnormally high free testosterone values were found in conjunction with inappropriately low LH, FSH values, normal response of LH and FSH to GnRH (Lamm *et al*. 2016). These anomalies occurred regardless of how long the patient had been hyperglycemic or how severe their condition was [glycosylated hemoglobin (HbA1c)]. In these individuals with hypogonadal state, magnetic resonance imaging did not reveal any abnormalities in the brain or pituitary gland. This link of HH with T2DM has already been verified in a number of medical investigations and is present in anywhere between 25 and 40 percent of these individuals. In light of this, the fact that the Endocrine Society now advises doing regular testosterone testing on individuals diagnosed with type 2 diabetes is an extremely significant development (Young *et al*. 2019).

It is proved that men with T2DM who have low testosterone levels also have a high prevalence of symptoms that are diagnostic of hypogonadism. These symptoms include easily becoming fatigued and having problems maintaining an erection (Rastrelli *et al*. 2015). The free testosterone values in addition to the total testosterone and were shown to have a negative relationship with both BMI and age in each of the aforementioned investigations. However, the existence of low testosterone concentration was not fully reliant on obesity since 25% of nonobese patients (31% of lean and 21% of overweight) also had HH (Grossmann 2018).

The percentage of patients who were overweight or lean did not significantly affect the prevalence of HH. Since HH occurs in only a small percentage of people with type 1 diabetes, it cannot be attributed to diabetes or hyperglycemia in and of itself. Therefore, considering the negative association that exists between BMI and testosterone values in people with type 1 and type 2 diabetes, it is likely that HH is linked with insulin resistance (Sizar and Schwartz 2021).

Previous research has shown a connection between hypogonadism and upper abdominal obesity, insulin resistance, and the metabolic syndrome. In males with T2DM, treatment with rosiglitazone for systemic insulin resistance results in a little rise in testosterone concentrations, however this does not result in normalization of testosterone concentrations (Surampudi *et al*. 2014).

1.1 Aim of Study

The levels of the role of copaptin in altering secondary hypogonadism in men with and without type 1and 2 DM are what will be investigated over the course of this research. The effects of copaptin and the metabolism will predominate as the primary topics of our conversation. In addition, we will talk about certain features of the physiology of copaptin and the impact it has on the metabolism of men who have Type 1 and Type 2 Diabetes as well as those who do not have either form of the disease.

In this study, there will be an evaluation of 120 patients (40 patients with type 1 diabetes and 40 patients with type 2 diabetes) with varying degrees of disease activity, as well as a control group of 40 healthy women (not pregnant) who will be compared to the patients in terms of the age, sex, and BMI. In men with and without type 1 and 2 diabetes, measurements of copaptin and other biochemical markers will be taken.

2. LITERATURE REVIEW

2.1 Hypogonadism

Problems in the gonadal response to gonadotropins or defects in the manufacture of sex hormones are the hallmarks of hypogonadism. Male hypogonadism (Figure 2.1), also known as a failure of the testes to generate enough values of testosterone or sperm, has become a frequent clinical finding, especially in the elderly population. This is especially true in countries where people are living longer. It is more probable that this is the consequence of an increase in knowledge and identification of the condition by medical professionals than than a genuine rise in the disorder's prevalence (Basaria 2014).

Before beginning therapy, it is necessary to verify and carefully examine the fact that the blood testosterone level is low. It is vital to ascertain whether the etiology is a main (hypergonadotropic) testicular problem or whether it is secondary to a process that involves the hypothalamus and the pituitary (hypogonadotropic or normogonadotropic). Hypogonadism is characterized by low levels of both free and total testosterone, in addition to sexual complaints (Sizar and Schwartz 2021). This insufficiency becomes more prevalent with age, which is a phenomenon that is both unique and difficult. The hypothalamic–pituitary–gonadal axis becomes deficient with age, which leads to a decline in testosterone production. This deficiency affects all three levels of the axis (Fraietta *et al*. 2013).

Studies showed that the testes are the organs that experience the most significant alterations, namely a reduction in the number of Leydig cells. In point of fact, men's testosterone values in their blood start to steadily decline between the ages of 40 and 50 at a rate of 1-2 percent every year when they reach that age range. The natural decline in testosterone production that occurs with aging is referred to as andropause or low-estrogen hormone (LOH). In adult males, testosterone has an important role in the maintenance of muscle mass and strength, fat distribution, bone mass, creation of red blood cells, the pattern of hair that men have, desire and potency, and the formation of spermatozoa (Young *et al*. 2019).

On the other hand, testosterone is the primary gonadal steroid hormone that is produced by males. Unbound testosterone, also known as free testosterone, tightly bound testosterone, also known as testosterone bound to SHBG, and weakly bound testosterone, also known as testosterone bound to albumin, are the three primary types of testosterones that circulate in the body. The only types of testosterones that are accessible or able to bind to the androgen receptor are free and weakly bound testosterone (Silveira and Latronico 2013).

There is a diurnal fluctuation in the amounts of testosterone found in the serum of men, with the levels being at their peak in the morning and their lowest in the afternoon and later. There is roughly a 35% margin of error in measuring testosterone levels in young males. Although the normal range for serum testosterone may change somewhat from one laboratory to another, the usual range for early morning total testosterone in healthy adult men is roughly 300 ng/dL to 1000 ng/dL prevalence. This range is based on the prevalence of testosterone in the blood (Lunenfeld *et al*. 2015).

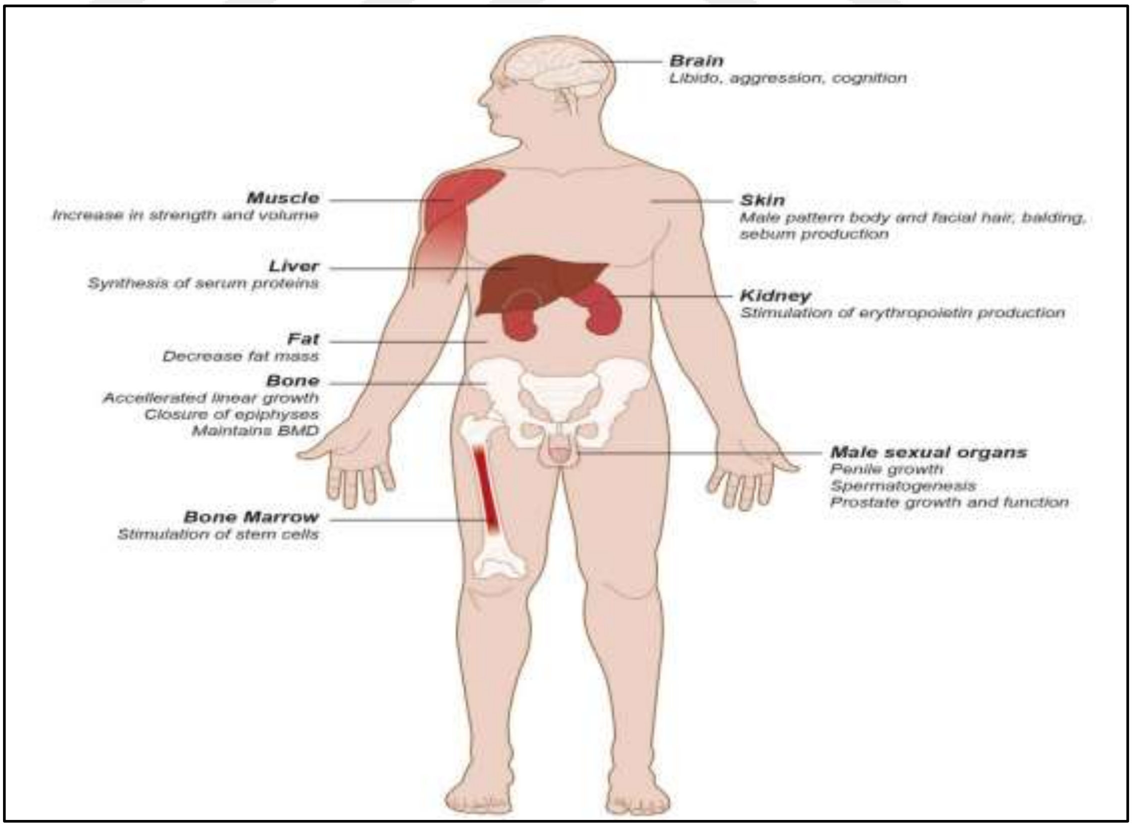

Figure 2.1 Male Hypogonadism (Sizar and Schwartz 2021)

2.1.1 Prevalence

Recent years have seen a rise in the prevalence of hypogonadism, which now affects 11% of men between the ages of 50 and 60, 19% of men between the ages of 60 and 70, and 49% of men between the ages of 70 and 80. Testosterone deficiency in men has been linked to a variety of signs and symptoms; the most essential symptom of testosterone insufficiency for a medical consultation is sexual dysfunction (Cangiano *et al.* 2021).

Decreased testosterone levels are often a borderline characteristic when it comes to the diagnosis of hypogonadism. The measurement of serum luteinizing hormone is another another criterion that may be used in the hypogonadism diagnostic process (LH). Through the Leydig cells, LH has a direct influence on the production of testosterone (Pivonello *et al*. 2019).

However, hypogonadism may be either primary (low testosterone, high LH) as a consequence of insufficiency of the testes or secondary (low testosterone, low or insufficiency LH) as a result of hypothalamic–pituitary insufficiency. Both forms of hypogonadism are summarized by low testosterone values. In reaction to the pulsatile release of LH, testosterone levels go through a variety of swings. Follicle-stimulating hormone, often known as FSH, has a lower pulsatility than LH, and a rise in its level suggests that the somniferous tubules have been damaged (Boehm *et al*. 2015).

According to the findings of several research, the symptoms of hypogonadism are linked to reduced levels of testosterone in the blood, both total and free testosterone. Other research, on the other hand, found no evidence of a substantial link between the symptoms of hypogonadism and the blood testosterone values. There does not seem to be a clear connection between the signs and symptoms of hypogonadism and the values of testosterone hormone, which results in differing points of view about the diagnosis, therapy, and management of the symptoms. In point of fact, the connection between hypogonadism and testosterone levels is an essential component in the process of diagnosing the condition, determining the best course of treatment, and evaluating the effectiveness for the therapy of the testosterone replacement (Dwyer *et al*. 2015).

2.1.2 Diagnosis

A doctor has to take into account the patient's clinical signs and symptoms in addition to the patient's laboratory data in order to evaluate whether or not the patient has a testosterone deficiency. The age of the patient at the time the disease first manifests itself will have a significant impact on the first clinical picture. The beginning of puberty in a typical man is characterized by the expansion of the testes as well as the emergence of hair in the pubic region, which is then followed by the growth of hair in the auxiliary areas and on the face. At puberty there is also increased penile length and the commencement of spermatogenesis. When a guy reaches the age of 14 and no indications of puberty have shown, it is necessary to investigate the possibility of delayed puberty (Cangiano *et al*. 2021).

At the start of puberty, LH and FSH are both released in the firt part of human day (early hours of the human morning); thus, it is imperative that these hormones be measured in the early morning hours. Primary hypogonadism is characterized by low levels of testosterone in addition to high-normal to high amounts of luteinizing hormone (LH) and follicle stimulating hormone (FSH). Secondary hypogonadism is characterized by low levels of testosterone in addition to normal or low levels of LH and FSH (Nieschlag 2020).

The values of testosterone, prolactin, FSH, and LH in the blood should all be measured first thing in the morning during the first laboratory testing. The measurement of FSH is especially significant for the diagnosis of primary hypogonadism since FSH has a longer half life, is more sensitive, and displays less fluctuation than LH. These may all be caused by testosterone deficiency. At the first appointment, the laboratory testing should include a measurement of serum testosterone taken first thing in the morning. Over the course of one day, the testosterone levels of older men drop by between 15 and 20 percent. In patients with hypogonadism, elevated SHBG levels may allow total testosterone levels to be within normal ranges. SHBG levels rise with age, which leads to a reduction in the amount of testosterone that is bioavailable (Ross and Bhasin 2016).

2.1.3 Development

The hypothalamic-pituitary-gonad axis is the most important regulator of sexual maturation and reproduction. These activities are triggered by the consisting of at least gonadotropin releasing hormone (GnRH). After a successful transition during embryo development of the GnRH neurons from the odorant placode, from across cribriform plate, and to the pituitary gland arcuate nucleus, where they are perceptible from about 9 weeks of gestation, GnRH is produced and released. This happens after the migration of the GnRH neurons successfully completes (Corona *et al*. 2015).

This hormone is secreted in a pulsatile manner and binds to its receptors on pituitary gonadotropes. In response, the pituitary gonadotropes then produce and release the gonadotropins LH and FSH. These attach to their respective corresponding receptors in the gonads, and once there, they promote the creation of sex hormones like androgens and estrogens, as well as the process of gametogenesis. At the level of the brain or pituitary, the sex steroids then control the gonadotropin release via a process known as negative feedback (Topaloglu and Kotan 2016).

Primary hypogonadism is the more common form of the disorder. Secondary hypogonadism is less common (secondary hypogonadism) (Nguyen *et al*. 2015). In primary hypogonadism, circulation quantities of LH and FSH are often elevated (hence the name hypergonadotropic hypogonadism), but testosterone and estrogen concentrations in men and females respectively are reduced. This condition is sometimes referred to as hypogonadotropic hypogonadism. Due to the low levels of circulating LH, FSH, testosterone, and estrogen that are typical of secondary hypogonadism, this condition is often referred to as hypogonadotropic hypogonadism (Rochira and Guaraldi 2014).

In addition to the 22 pairs of autosomal chromosomes, human beings have one pair of sex chromosomes, which are either XY or XX depending on the gender. The presence or absence of the Y-chromosomal SRY gene, also known as the sex determination region of the Y chromosome, has a significant impact on the development of the embryonic bipotential gonad, which is so termed because it has the potential to develop into either the male testis or the female ovary (Salonia *et al*. 2019).

Once this process is started, a complicated chain reaction of morphological changes and genetic control takes place, which ultimately results in the formation of male sexual traits. This gene functions as a switch that encourages the bipotential gonad towards developing the testis. In the absence of the Y chromosome and, as a consequence, this genetic flip, a different chain reaction of morphological changes and genetic regulation takes place, which ultimately results in the development of the ovary and the acquisition of female sexual traits. These primitive sex cords are necessary for normal sexual development (Richard-Eaglin 2018).

These, together with their progenitors in the mesenchyme, are the cells that will eventually become the theca or the Leydig cells that secrete steroids. The primordial germ cells begin to infiltrate and multiply in the primitive sex cords during the sixth week of gestation. These sex cords will continue to foster the primordial germ cells as they mature. As the preceding processes take place, primitive Wolffian and Müllerian ductal networks may be seen running parallel to one another along the surface of the mesonephros or genital ridge. These ductal systems will eventually develop into the male and female ductal systems, respectively. After the initiation of the sex determination process, only one of these will continue to grow to its full potential, while the other will regress (Forni and Wray 2015).

This process takes place in male embryos; when the primordial germ cells stop dividing and go into mitotic arrest, they are referred to as gonocytes or prespermatogonia. They continue to be largely dormant until puberty, when they become dependent on the Sertoli cells for nutrition and structural support during the process of spermatogenesis. The production of AMH by Sertoli cells, which occurs with formation of the early seminiferous tubules, inhibits the development of the female lineage and is hence essential for male fertility (Khera *et al*. 2016).

Following this, the size of the primitive testis expands as a result of the proliferation and migration of underlying mesonephric mesenchymal cells. These cells are responsible for the production of peritubular myoid cells, which are important contributors to testicular vasculature, as well as steroidogenic Leydig cells. These cells are not allowed to enter the seminiferous tubules and instead make up the interstitium, which is the second compartment of the testis (Zarotsky *et al*. 2014).

The synthesis of testosterone by Leydig cells may be observed as early as 8 weeks of gestation and reaches its peak between 12 and 14 weeks. The hypothalamic GnRH and pituitary gonadotropins are not responsible for the stimulation of testosterone production since these hormones are not formed beyond 8 weeks. Instead, the stimulation is brought about by the LH analogue known as hCG, which is found in the placenta (Huhtaniemi 2014).

The hormone testosterone does not have an especially strong affinity for the androgen receptor on its own. Instead, the enzyme known as 5-alpha-reductase, which is found on the surface of cells, converts it into the considerably more powerful androgen known as dihydrotestosterone (DHT). In point of fact, virilization is incomplete in a fetus that lacks DHT (Amato *et al*. 2019).

2.2 Copeptin

Copeptin is a glycopeptide, this kind of a glycopeptide most probably includes of 39 amino acids that makes up the C-terminal portion of the arginine-vasopressin (AVP) precursor (pro-AVP). It is produced in the same stoichiometric manner as AVP and is released into the bloodstream at the same time. As a result, it accurately mirrors the presence and activity of AVP. In the hypothalamus, the magnocellular neuronal nuclei of the paraventricular and supraoptic regions are functional of the synthesis of pro-AVP. During the process of axonal transport towards the pituitary gland, an enzymatic cleavage takes place, which not only assures the production of the two mature molecules but also separates them from their carrier, neurophysin II. In response to hemodynamic and osmotic stimuli, AVP is then produced with copeptin from the posterior pituitary through into systemic circulation. This is done in order to maintain vascular tone (receptors V1a, V1a-R) and fluid homeostasis (V2-R) (Dobša and Cullen 2013).

Since its first characterization was as a region had the highest from the posterior pituitary that was completely void of just about any pituitary-related bioactive components other than a subtle lipolytic effect, copeptin has yet undiscovered functions: it was initially, but unsatisfactorily proposed as the prolactin-releasing factor, while more recently it has been highlighted as a chaperone protein that plays an important role in the appropriate folding and proteolytic maturation of AVP (Figure 2.2). On the other hand, copeptin decay kinetics hints at the presence of additional specific peripheral operations, even in the absence of identified specific receptors or elimination mechanisms (Maisel *et al*. 2013).

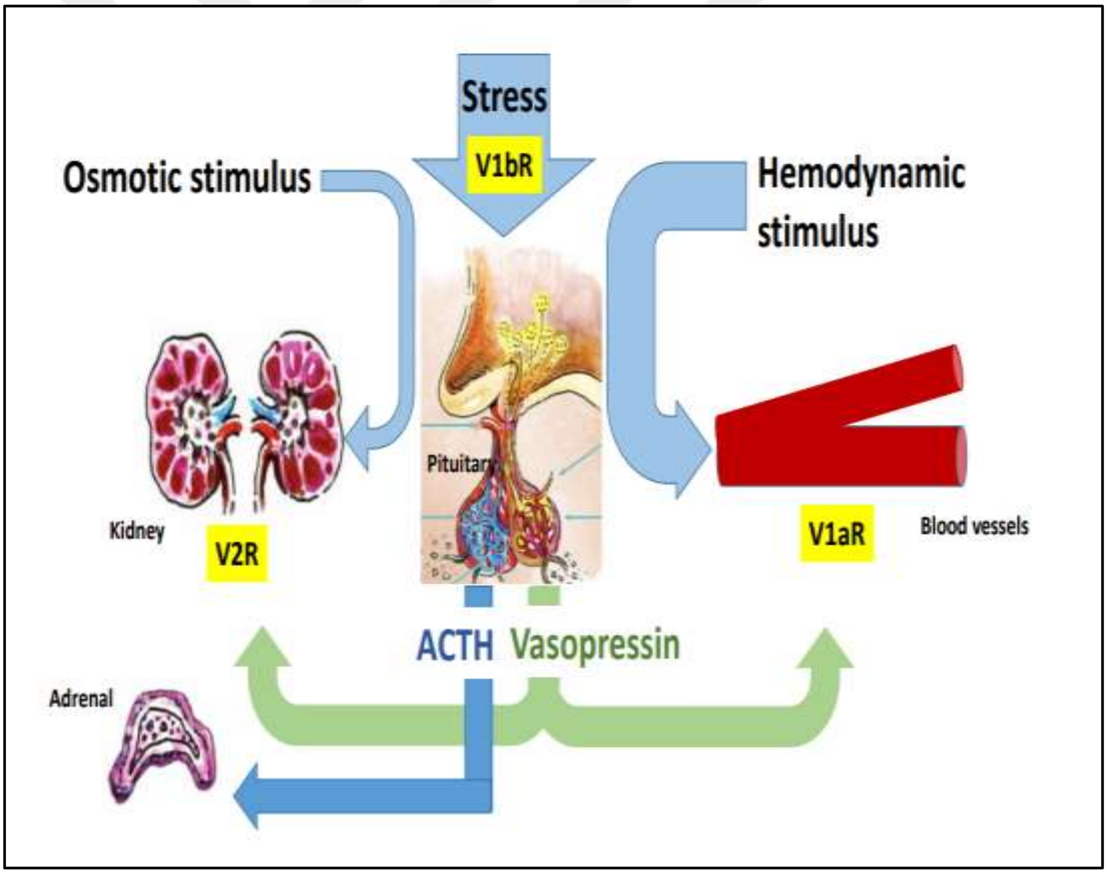

Figure 2.2 Regulation of AVP and the release of copeptin (Fenske *et al*. 2018)

2.2.1 Measurements

In order to evaluate copeptin, you won't need to go through any complicated pre-analytical activities. One has the option of manually measuring it or using completely automated tests. In addition, in contrast to measures of vasopressin, copeptin only requires a tiny volume of plasma (approximately 50 microliters, as opposed to 400 microliters for vasopressin). Because of all of these considerations, measuring copeptin may serve as a suitable substitute for the normal measurement of vasopressin (Morawiec and Kawecki 2013).

There are a few different copeptin tests that may be performed, of which only two are of high enough quality to be approved for clinical usage within the. It is a one-step test that utilizes coated tube technology. There is one antibody that is linked to the tubes (polystyrene), and another antibody that is tagged for chemiluminescence detection. The minimum quantity that may be detected is 0.4 pmol/l, and the FAS is less than 1 pmol/L (Yalta *et al*. 2013).

Another technique, known as time-resolved amplified cryptate emission, is used in this method. It lengthens the light from the proper signal as opposed to going through washing and separation phases in order to get rid of background noise. This approach has a detection limit of 0.7 pmol/L for analytical purposes, and the FAS is less than 1.08 pmol/L. When compared to the incubation period of CT-proAVP LIA, which is two hours, this method only takes 14 minutes. Therefore, drinking fluids induces a quick decrease in levels, while a rise in levels is caused by thirst in healthy cohorts (Urwyler *et al*. 2015).

Copeptin is a potential prognostic marker in a number of medical disorders that cause significant amounts of stress, such as acute myocardial infarction (acute MI). In 101 critically sick patients, the levels of copeptin showed a substantial upward trend with increasing illness severity. Sik individuals diagnosed with sepsis had a median copeptin value of 50.0 (interquartile range (IQR): 8.5-268) pmol/L; patients diagnosed with severe sepsis had 73.6 (IQR 15.3-317) pmol/L; and patients diagnosed with septic shock had the highest levels, (IQR: 35.1-504) pmol/L. Researchers have shown a correlation between rising levels of copeptin and the severity of lower respiratory tract infections as well as their outcomes (De Marchis *et al*. 2013).

Additionally, copeptin research has been conducted in the context of various manifestations of cardiovascular diseases (CVDs), as will be further detailed below, and then in particular with a concentration on risk prediction. In individuals who had had an ischemic stroke, copeptin accurately predicted both functional prognosis and death after three months. At admission, the median value of copeptin recorded about 14.2 pmol/L, with an interquartile range of 5.9 to 46.5. In addition, copeptin was able to accurately predict recurrent vascular events in individuals who had previously had a stroke or a transient ischemic attack (Bolignano *et al*. 2014).

2.2.2 Relation with cardiovascular diseases

Because AMI is in relationship with an abrupt rise in copeptin values, followed by a rapid fall in those levels, copeptin has emerged as a potentially useful clinical diagnostic for excluding AMI. Vasopressin activation, which was assessed as copeptin, was detected in two separate cohorts during the acute phase of MI. Some previous studies reported the highest mean level of copeptin, 21.8 μM/L, upon admission, with a rapid drop sometimes to 8.5 μM/L at the time of hospital discharge, four to five days after admission. Despite this, the level is still high compared to what has been reported in healthy individuals (average 3.8-6.0 pmol/L). These findings are consistent with the findings of the LAMP trial, which included a total of 980 patients and 700 controls. During the first five days after their AMI diagnosis, 132 patients who were participating in the LAMP trial provided daily blood samples for analysis (Christ-Crain 2019).

When compared to days two through five, the copeptin levels were considerably higher on day one (p 0.001), but they leveled out quite rapidly between days three and five. The amounts of copeptin that were tested in the plateau continued to be greater than the concentrations in the control cohort (7.0 vs 3.8 pmol/L, p 0.001). However, all of the sick individuals had T2DM, but in the LAMP study, only around one fifth of the patients had (Velho *et al*. 2013).

This is one of the key differences between the two studies. It's possible that this had an effect on the copeptin levels. As was just noted, the measurement of copeptin took place at the time of hospital admission in Study I, while samples were collected the morning after hospitalization. In addition to this, it is important to take into account the disparities that exist across the patient cohorts. Patients participating in this investigation were older, had a higher of BMI, and had a history of high blood pressure and heart failure that was more prevalent (Enhörning *et al*. 2015).

Whereas the rise of copeptin was, in point of fact, much more pronounced for patients who were diagnosed with AGT during their hospital stay than it was for individuals who had NGT. As a result, the presence of dysglycemia seems to be connected with greater levels of copeptin and may have repercussions for the vasopressin response (Balmelli *et al*. 2013). The patients who took part had copeptin levels that were 10.5 pmol/L, which is much lower than the levels seen (21.8 pmol/L). Different phases of dysglycemia are one probable reason for this phenomenon. In Study I, all of the patients had type 2 diabetes, which was an exclusion criterion, in which 31% of the participants had NGT and the remaining patients had newly discovered AGT based on an OGTT. One other option is the varying times at which copeptin levels were measured (Yeung *et al*. 2014).

The increase or decrease in plasma osmolarity concentrations in addition to the circulating volume acting through baroreceptors is the primary driver of vasopressin production. It is well known that copeptin responds swiftly to changes for both of the fluid status in addition to the plasma osmolality. As a result, one possible explanation for the elevation of copeptin that occurs during AMI is that it is a reflection of the vasopressin activation that occurs as a secondary effect of the hemodynamic shift. This shift most probably caused by an occlusion of CA, which causes an increase in left ventricular end-diastolic pressure and a decrease in blood pressure, and it may also cause the baroreceptors to be activated (Zhong *et al*. 2017).

Furthermore, the results were expanded upon by additional research that showed an increased level of copeptin in people who had a prior MI. This indicates that the patients were in a more stable phase, which suggests a continuous activation of vasopressin. Even after the acute stressor has been removed from the body, an elevated level of copeptin is a reflection of the chronic burden of stress. It's possible that this has anything to do with the underlying stress that results from cardiac remodeling caused by myocardial injury. This prolonged stimulation of vasopressin may potentially result in more harm, for instance; heart failure through the healing phase; this is something that will be further examined in the thesis (Tasevska *et al*. 2016).

However, the activation of the vasopressin receptors might potentially have negative consequences on both the cardiovascular system and the heart. Vasopressin may have pro-thrombotic effects by increasing the release of von Willebrand factor (vWF) and Factor VII from endothelial cells through activation of endothelial V2-receptor. These effects may be especially damaging for individuals who have had an AMI. In addition, activation of vasopressin has several effects, each one of which may contribute to the remodeling that occurs after a MI. Left ventricular hypertrophy may result from the increased protein synthesis of myocytes that is caused by the activation of the V1areceptors (Balling and Gustafsson 2014).

Additionally, an increase in afterload may occur as a result of the activation of V1a-receptors on vascular smooth muscle cells, which causes vasoconstriction. In addition, activation of V2-receptors may cause an increase in blood volume, which can ultimately result in an increased risk of heart failure (Raskovalova *et al*. 2014). Increased copeptin concentrations, when evaluated three to five days after an AMI, were shown to correlate negatively with left ventricular ejection fraction and remodeling five months later in research that was carried out by Kelly and colleagues. In addition, the association between copeptin and the extent of the MI was studied in research that included 54 individuals with ST-elevation myocardial infarction (Möckel and Searle 2014).

Other investigations found that the levels of copeptin were greater than in healthy cohorts (3.8 to 6.0 pmol/L), and this was the same regardless of whether or not the MI had previously been known to the participants. This suggests that both RMI and UMI have a comparable level of vasopressin activation, which is a conclusion that is supported by the fact that their respective prognoses are comparable. Age may also play a part, seeing as included participants who were old (Timper *et al*. 2015).

The influence of aging on copeptin, on the other hand, has not been well studed. The link between the marker and thecopeptin the of heart failure known as NT-proBNP was investigated in order to determine whether or not elevated levels of copeptin in individuals with coronary artery disease (CAD) may be linked to heart failure and myocardial dysfunction. Although there was a substantial correlation between copeptin and NT-proBNP, the association between copeptin and CVE, MI, and overall mortality persisted after adjusting for NT-proBNP (Roussel *et al*. 2014).

2.2.3 Relation with deglycation and T2DM

It wasn't until 1979 that an increased level of vasopressin in diabetes was first documented; since then, it's been proposed that it plays a role in dysglycemia. High levels of copeptin were seen in patients with established T2DM at the time of admission for AMI as well as three months later. This was previously highlighted (Evers and Wellmann 2016). When compared to what has been observed in other studies including mixed cohorts of patients with AMI, the median admission level was much higher. This was reinforced by Study II, which found that patients with AMI and newly diagnosed AGT had considerably greater levels of copeptin than those patients with NGT (Lukaszyk and Małyszko 2015).

A multinational investigation that included many centers validated these results by enrolling 370 patients with diabetes (type 1 and type 2) as well as 1621 individuals without diabetes who were hospitalized to the emergency room with symptoms that were indicative of an AMI. Regardless of the underlying reason of the patient's chest discomfort, the copeptin levels that were tested at the time of admission were greater in diabetic patients than in individuals who did not have diabetes (10.4 vs. 6.2 pmol/L: p 0.01). This leads one to believe that the rise in copeptin that occurs after an AMI, or maybe CAD in general, is not just connected to the occurrence of CAD in individuals who have diabetes (Mueller *et al*. 2018).

Diabetes on its own seems to be linked to higher copeptin levels and, as a result, increased vasopressin (Figure 2.3) activation, which may reflect a greater susceptibility to stress. Patients who have type 2 diabetes already have an underlying chronic condition, which may result in greater vasopressin activation during the acute phase of an acute myocardial infarction (AMI). It is possible that this makes them more sensitive to the stress that is produced by an AMI. In unadjusted analyses, the level of copeptin that was assessed in sick people with AMI in addition to AGT were recorded a significant predictor for CVE. This was not the case for patients with NGT, however (Afsar 2017).

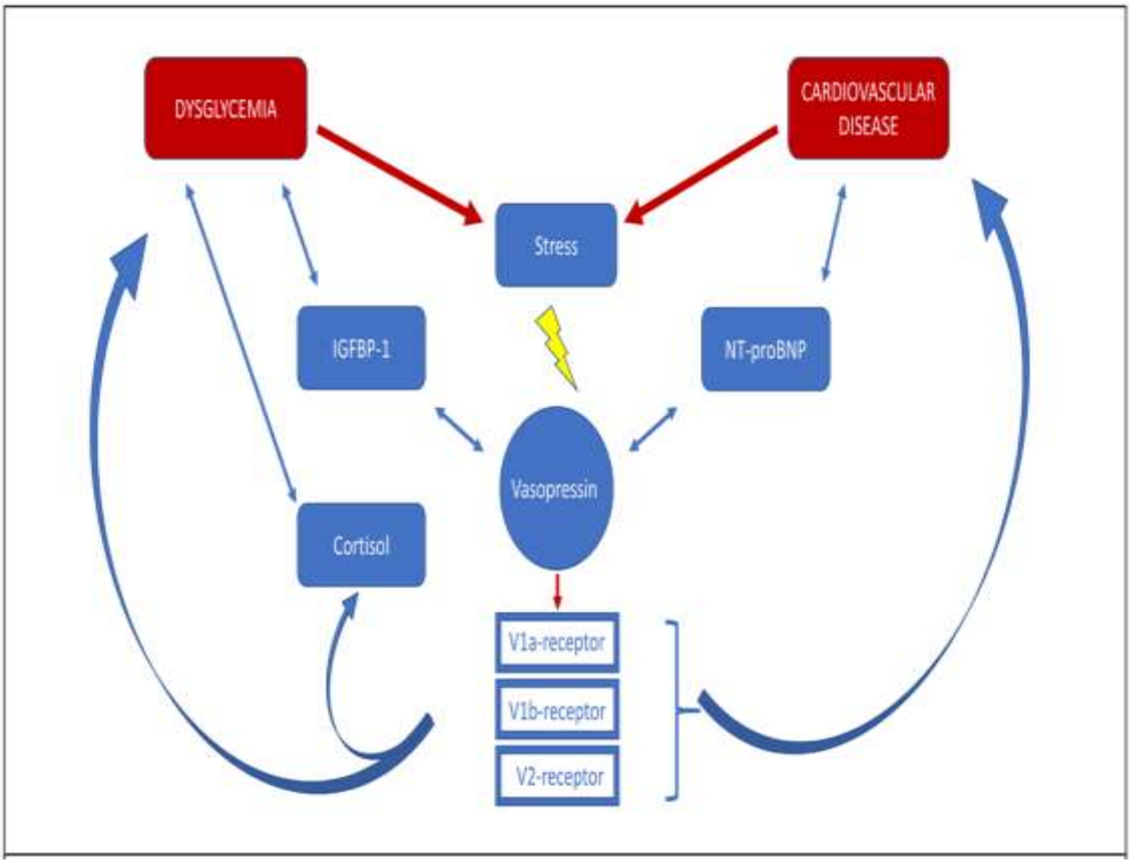

Figure 2.3 A potential relation for vasopressin, CVD, and dysglycemia (Refardt *et al*. 2019)

2.3 Diabetes Mellitus

Diabetes mellitus, more often refers to as diabetes, is a set of disorders that are characterized by high blood glucose values. These high blood glucose values are the consequence of deficiencies in the body's capacity to generate and/or utilize insulin. Diabetes may also be referred to as just diabetes (McIntyre *et al*. 2019).

It is a condition that is largely characterized by the degree to which hyperglycemia raises the risk of harm to the microvascular system (retinopathy, nephropathy and neuropathy). It is linked to a shorter expected lifespan, considerable morbidity owing to certain microvascular disorders that are associated with diabetes (Blair 2016).

There are two types of DM (Figure 2.4) and a number of different pathogenetic pathways that play to the implementation of diabetes. The presence of diabetes mellitus may be indicated by several symptoms, including dry mouth, increased urination, blurred eyesight, and decreased body weight. In many cases, the symptoms are mild or may even be completely absent (Kharroubi and Darwish 2015).

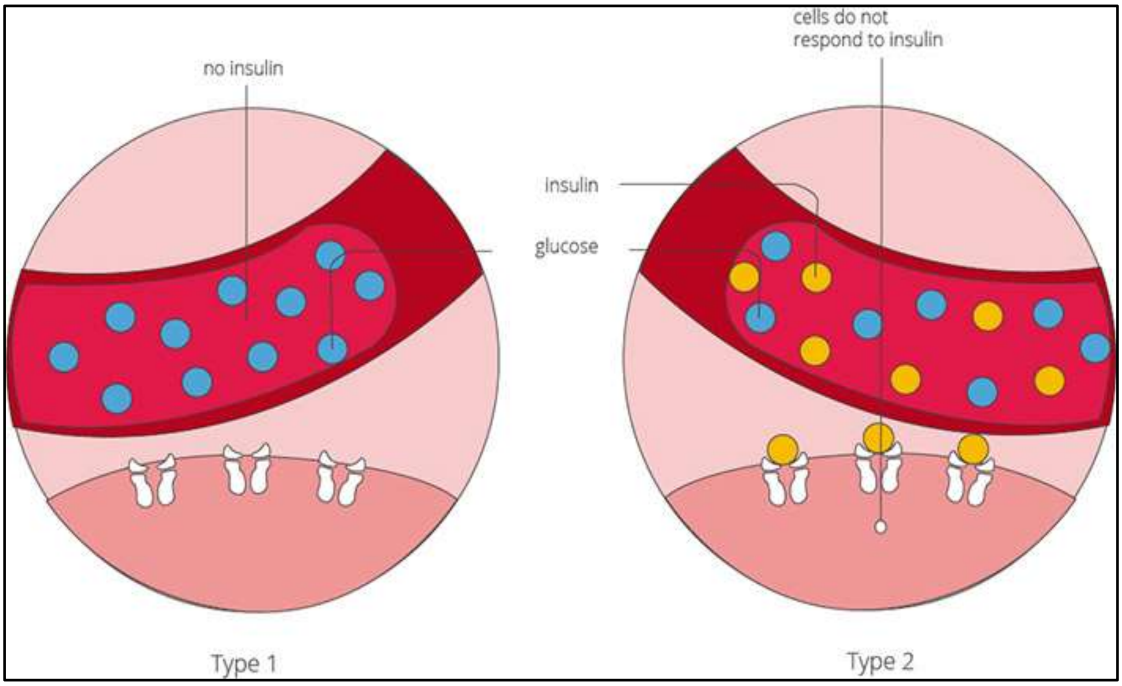

Figure 2.4 Types of DM (Cole and Florez 2020)

2.3.1 Pathophysiology

Knowledge of the fundamentals of glucose metabolism and the action of insulin is necessary for gaining a grasp of the pathophysiology behind diabetes. Following the ingestion of food, the digestive tract is responsible for the breakdown of carbohydrates into glucose molecules (Galicia *et al*. 2020).

The levels of glucose in the blood rise as a result of the absorption of glucose into the circulation. This increase in glycemia prompts the beta cells in the pancreas to start producing more insulin as a response (Gromada *et al*. 2018).

Insulin is required by most cells to enable glucose access. Insulin is able to attach to certain cellular receptors, which in turn makes it easier for glucose to enter the cell and be used as a source of energy by the cell. Blood glucose levels drop as a result of greater insulin secretion from the endocrine and subsequent glucose consumption by cells (Buczkowska and Jainta 2018). This process causes the pancreas to produce more insulin. Lower glucose levels then result in lower insulin secretion (Coustan 2013).

If you have an illness that affects insulin synthesis and secretion, the dynamics of your blood glucose will also shift. Hyperglycemia will develop if insulin production is lowered, since this will prevent glucose from entering cells and lead to the condition (Padhi *et al*. 2020).

If insulin is released from the pancreas but target cells do not utilize it correctly, the same impact will be observed. If insulin is secreted in greater quantities, blood glucose levels may drop to dangerously low levels, a condition known as hypoglycemia. This occurs when large levels of glucose reach tissue cells, leaving just a little quantity in the circulation (Plows *et al*. 2018).

2.3.2 Complications

Diabetes-related complications are a major contributor to the development of disabilities, a decline in death and quality of life. A bad effects of diabetes may affect a variety of sections of the body and present in a variety of unique ways for each individual who has them. Sick individuals with diabetes have a significantly increased risk for a variety of major health complications (Ginter and Simko 2013).

It is accountable for erectile dysfunction, weak testosterone concentrations, and emotional issues in males for instance; depression, anxiety, or stress that may interfere with sexual emotions. In female, it is responsible for impotence and low testosterone concentrations (Kharroubi and Darwish 2015). Diabetes may be a particularly challenging condition for women. Even in those who have never had diabetes before, developing gestational diabetes is a possibility during pregnancy. Women who have diabetes have a significantly increased risk of dying from heart disease (Deshmukh *et al*. 2015).

In addition, women who have diabetes are more likely to suffer from depression, their sexual health may be compromised, and eating disorders are more likely to play a factor in their lives. Diabetes may cause complications in any region of the body, including the eyes, foot, and skin. In point of fact, these kinds of issues are often the very first indicator that a person suffers from diabetes. Complications with the foot have the potential to worsen and lead to more significant conditions for instance neuropathy, skin changes, calluses, and foot ulcers, as well as impaired circulation (Sowers 2013).

2.3.3 Diagnosis

The following examinations are used in order to arrive at a preliminary diagnosis: A test called a FPG exam checks the value of glucose in the blood of a person who has gone at least 8 hours without eating anything. Both diabetes and its precursor, prediabetes, may be identified using this test (Deshmukh *et al*. 2015). An oral glucose tolerance test, often known as an OGTT, involves measuring a person's blood glucose levels after they have fasted for at least 8 hours and again 2 hours after they have consumed a beverage that contains glucose. Both diabetes and prediabetes may be identified with the use of this test. The FPG test is the one that is most often used to diagnose diabetes due to the fact that it is both convenient and inexpensive (Chiefari *et al*. 2017).

On the other hand, it is possible that it will overlook certain cases of diabetes or pre-diabetes that may be detected by an OGTT. The FPG test is at its most trustworthy when performed first thing in the morning. When it comes to detecting prediabetes, research has revealed that the OGTT is more sensitive than the FPG test; nevertheless, the administration process for the OGTT is more difficult. Blood glucose is measured using what is known as a random plasma glucose test or a casual plasma glucose test (Sagarra *et al*. 2014).

This kind of test does not take into consideration the individual's most recent meal. Diabetes, but not prediabetes, may be diagnosed with the use of this test when combined with an evaluation of the symptoms. It is recommended that a second test be performed on a separate day if the findings of the first test suggest that a person has diabetes (Dörhöfer *et al*. 2013).

2.3.4 Types

Two main catagorizations of DM were proposed: IDDM (Type I) and NIDDM (Type II). Type 1 DM only accounts for roughly 5–10% of all instances of diabetes; nonetheless, its occurrence is continuing to grow globally, and it has major consequences both in the near term and in the long term (Fink *et al*. 2019). Type I denotes the process of beta-cell destruction in the pancreas, which can ultimately lead to DM, a condition in which "insulin is required for survival" to prevent the implementation of ketoacidosis, coma, and death. Type II denotes the absence of beta-cell destruction in the pancreas (Deuschle 2013).

The management of diabetes type I is best carried out within the context of a multidisciplinary health team, and it requires continuous attention to a wide variety of aspects, such as the administration of insulin, the monitoring of blood glucose, the planning of meals, and the screening for complications related to diabetes. These problems include microvascular and macrovascular disease, which together are responsible for the majority of the morbidity and death that is associated with type I diabetes (Kostoglou *et al*. 2013).

The most common kind of diabetes is called type II diabetes. Diabetes type II has been identified in the medical histories of millions of people all around the globe, and the condition is likely present in many more individuals who have not yet been tested (Oguntibeju 2019).

People who have diabetes at a larger risk of having cardiovascular disorders including heart attack and stroke if the condition is not recognized in a timely manner or if it is not managed well. A loss of vision, renal failure requiring dialysis or transplantation, amputation of the foot and leg owing to damage to the nerves and blood vessels, and amputation of the foot and leg due to damage to the blood vessels are all among the increased risks associated with this condition (Dörhöfer *et al*. 2013).

Individuals nearly always have "prediabetes" before they acquire Type II diabetes, which refers to blood glucose levels that are higher than usual but are not yet high enough to be identified as diabetes. This condition occurs before people develop diabetes. Recent studies have demonstrated that prediabetes may already be causing some long-term harm to the body, particularly to the cardiovascular system and the heart. Either the body does not create enough insulin or the cells in the body do not respond to the insulin. This results in type II diabetes. Insulin is essential for the body to have in order for it to be able to use glucose as a source of energy (Xiang *et al*. 2018).

Following the digestion of meals, the body converts all of the sugars and starches into glucose, which serves as the primary source of fuel for the body's cells. Insulin is responsible for transporting sugar from the blood into the cells of the body. Complications associated with diabetes might arise when glucose remains in the bloodstream rather than being absorbed by the cells (Chiefari *et al*. 2017).

Individuals nearly always have prediabetes before they acquire Type II diabetes, which refers to blood glucose concentrations that are higher than usual but are not yet high enough to be identified as diabetes (Ginter and Simko 2013).

This condition occurs before people develop diabetes. The medical condition known as prediabetes is a significant one that may be addressed. People who have prediabetes have a better chance of avoiding developing type 2 diabetes if they modify their eating habits and get more exercise, according to the findings of a study that was just finished and published by researchers in the United States. They could even be able to return their blood glucose levels to the normal range with the right treatment. Modifications to one's way of life are of the highest significance (Katsarou *et al*. 2017).

It is possible to keep a healthy weight, remain healthier for a longer period of time, and lower one's risk of developing diabetes via the adoption of a balanced diet and an increase in the amount of physical activity. The findings demonstrated that a reduced risk of developing Type II diabetes may be achieved via modest dietary modification and increased physical activity. Major clinical research study being conducted at multiple centers to determine whether or not a moderate loss of weight brought on by alterations to one's diet and an increase in the amount of time spent engaging in physical activity, or treatment with the oral diabetes medication metformin (Glucophage), could prevent or delay the onset of type II diabetes in those who participated in the study (Paschou *et al*. 2018).

2.4 Insulin and Diabetics

Insulin is a hormone that is used to treat diabetes. Insulin works by regulating how much sugar (glucose) is present in the blood. When it is prepared for medical use, it is either taken from pigs (porcine), cattle (which is not currently accessible in the United States), or it is genetically engineered to be genetically similar to human insulin. Diabetes, which is one of the leading reasons for rising cardiovascular morbidity and death in Western nations, places a significant burden on health care systems, both directly and indirectly, in terms of the expenses that are incurred. Therefore, effective glucose management, which is defined as the achievement of normal levels of HbA1C, prandial glucose, and postprandial glucose, is critical for the avoidance of the existence complications that may arise from this condition (Kotwal *et al*. 2019).

Since the hormone is no continuously generated internally, patients with diabetes mellitus type I are reliant on insulin from the outside world for their continued existence. This insulin is most usually administered subcutaneously. Insulin comes in a wide variety and is utilized to treat diabetes in many different ways. They are categorized according to how quickly they begin to exert their effects, at what point in time they reach their "peak" level of action (i.e., when the amount of insulin present in the blood is at its highest), and for how lengthy their impacts continue to be effective (Stamatouli *et al*. 2018).

The injection of insulin for diabetes may be done either subcutaneously (under the skin) or intravenously (in the vein) (intravenously). Subcutaneous insulin injection is still the basis of treatment for all persons who have diabetes mellitus type I and the majority of people who have diabetes mellitus type II. This is because insulin is absorbed more effectively via the subcutaneous route. Injecting insulin can be done with a syringe and needle or with a cylinder system, or with prefilled pen systems (Heller *et al*. 2016).

The first dosage is determined by the patient's weight and susceptibility to insulin, which varies from individual to individual. This information is used to make a calculation. Insulin is normally administered subcutaneously such that two-thirds of the entire daily dosage is given in the morning, and one-third of the daily total dose is administered in the evening. This schedule is followed in order to maintain blood sugar levels at a healthy level (Schlesinger *et al*. 2013).

It has been hypothesized that strict glycemic control in conjunction with intensive insulin treatment (Figure 2.5) may be able to lower the incidence of such problems in a number of diabetic populations. On the other hand, such a strategy may also be linked with a number of hazards and obstacles. Hypertrophy (an expansion of the area of the body that has absorbed an excessive amount of insulin injections), rash at the injection location or all over the body, and hypoglycemia (a drop in blood sugar) are the primary adverse reactions that may occur when insulin is used to treat diabetes (rare) (Chiefari *et al*. 2017).

Extreme hunger, exhaustion, impatience, cold sweats, shaky hands, severe anxiety, and a general feeling of disorientation are some of the symptoms associated with the most prevalent consequence, which is low blood sugar (Heller *et al*. 2016). Other symptoms include shaking hands. It's possible that these are the warning indications of an insulin excess, a potentially life-threatening complication of diabetes that affects a significant number of diabetic people. Patients who adhere to a few basic guidelines may, thankfully, prevent the majority of insulin-related complications from occurring (Perdigoto *et al*. 2019).

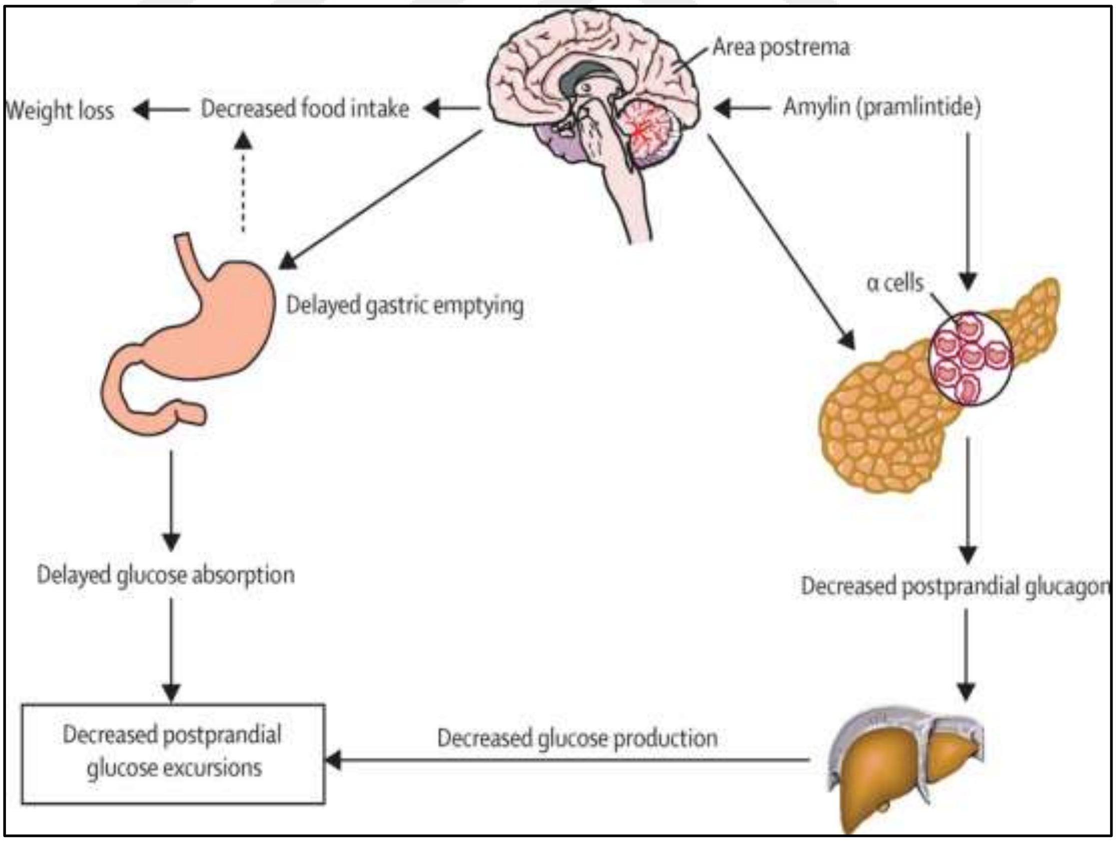

Figure 2.5 Insulin process to treat DM (Heller *et al*. 2016)

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Tools

Some of the medical devices and other laboratory materials are summarized in Table 3.1.

Table 3.1 Important material, devices, and tools during this current study

Devices and tools	Origins
Water distilled	GFL, Germany
Water path	Memmert, Germany
Centrifuge	Hettich, Germany
Refrigerator	Samsung, Korea
Incubator	Korea
Mercantile automated immuno fluoresencs assay	BRAHMS CT-pro AVPLIA, Gmbll, Germany
Precision pipette 50 µL, 100 µL, 1 mL	China
Disposable pipette tips	China
Vortex mixer	UK
Microtiter plate readre	UK

3.1.2 Patients chosen and the studied groups

The roles for the chosen of patients to participate in this study were classified according to Wagner classification and informed consent taken from the patient to make the study, they were allocated randomly to the groups. According to study objective and to do enhance results. In the experments in the lab within the field of this study, 40 samples of patients with DM1, 40 samples of patients with DM2 and healthy control (40 person) were chosen and divided into three groups, these groups of the present study are clearly shown below:

Group A: Consists of 40 patients with DM1.
Group B: Consists of 40 patients with DM2.
Group C: Consists of 40 healthy subjects without diabetes disease.

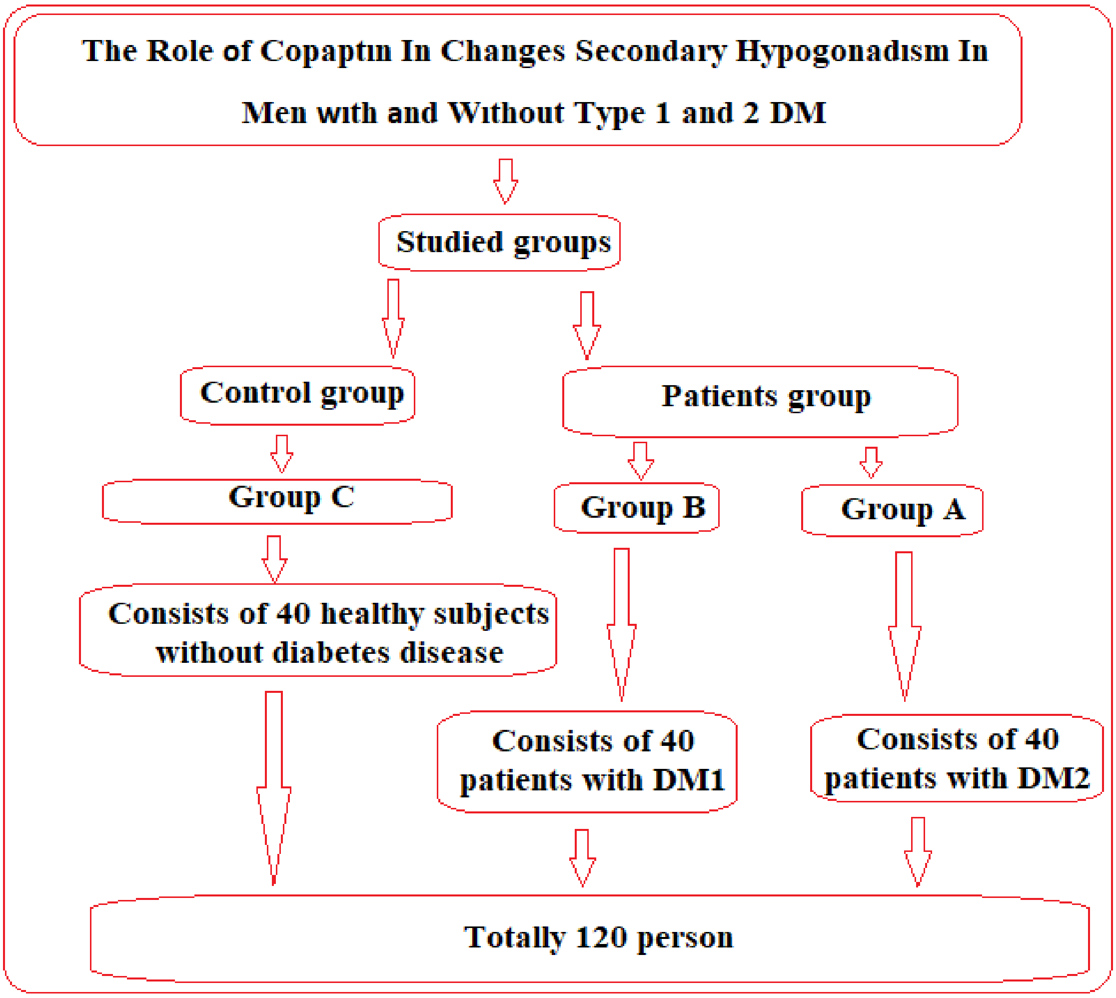

Figure 3.1 Studied groups

3.1.3 Blood samples

The blood samples were collected and determined the biochemical measurements for the role of copeptin in changes secondary hypogonadism in men with and without Type 1 and 2 DM.

3.1.4 The aim of this study

The aim of current medical jub is to study the efficiency of copeptin in changes secondary hypogonadism in men with and without Type 1and 2 DM and we will focus our discussion primarily on the effects of copaptin and metabolism. In addition, we will discuss some aspects of the physiology of copaptin and the effects it on metabolism in Men with and without Type 1and 2 diabetes mellitus.

3.2 Methods

3.2.1 Total cholesterol (Tc) test

The examination for the measurement the total cholesterol in blood sample comprises three enzymes, cholesterol esterse, cholesterol oxidase and peroxidase, see Equation (3.1), Equation (3.2), and Equation (3.3). Cholesterol esterase enzyme transformed to cholesterol and fattey acids, and the cholesterol is oxidate to cholestenone and hydrogen peroxide and finally the mixture of phenol and 4- aminoantipyrine are intensified by hydrogen peroxide to constitute quinonemine dye and H_2O, the quinonemine dye is symbolizes to the choesterol concentration in the sample.

$$\text{Cholesterol esterase} \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol + Fatty acids} \quad (3.1)$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholestenonemine} + H_2O_2 \quad (3.2)$$

$$\text{4- AA –Phenol} \xrightarrow{\text{Peroxidase}} \text{Quinonemine} + 4H_2O \quad (3.3)$$

Assay

Add 1 mL of from blank, sample and cal standard, then put it in R1 monoreagent tube, put 10 µL of sample in sample tube and put 10 µL of cal standard in cal standard tube. Incubate it at 25 ºC for 10 minutes, and finally read the absurbance at 500 nm against the blank.

3.2.2 Total triglyceride (Tg) test

This assay is based on hydrolysis of serum triglyceride to form glycerol and fatty acids by aiding the enzyme lipoprotein lipase, the glycerol in phosphoglycerated by aiding the ATP in exist of glycerol kinase with genesis glycerol-3- phosphate and ADP. Glycerol - 3- phosphate is oxidized with origination DHAP and hydrogen by the aiding the glycerophosphate oxidase. By assist the enzyme peroxidase 4- aminoantipyrine and phenol are stimulated with forming quinoemine and water, proportional to triglycerides concentration in the sample and its calculation described in Equation (3.4), Equation (3.5), Equation (3.6), in addition to the Equation (3.7).

$$\text{Triglycerides} + 3H_2 \xrightarrow{\text{Lipoprotein lipase}} \text{Glycerol} + 3\text{FFA} \tag{3.4}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycero kinase}} \text{Glycerol-3- P} + \text{ADP} \tag{3.5}$$

$$\text{Glycerol-3- P} + O_2 \xrightarrow{\text{Glycerophosphate oxidase}} \text{DHAP} + H_2O_2 \tag{3.6}$$

$$\text{4-AA} + 4\ \text{Phenol} \xrightarrow{H_2O_2\ /\ \text{peroxidase}} \text{Quinnonemine} + H_2O \tag{3.7}$$

Assay

Add 1 mL of blank, sample and cal standard and put it in R1 monoreagent tube and mix well, then 10 µL of sample was added in sample tube, also 10 µL of cal standard was added in cal standard tube. Let the tubes stand for 15 minutes at 25°C, finally read the absurbance of the samples at 500 nm. For calculations of triglycerides, see Equation (3.8).

$$\{\text{A sampls-A standard}\} \times \text{C Standard} = \text{mg /dL triglycerides} \tag{3.8}$$

3.2.3 High density lipoprotein (HDL) test

Principle

This examination is based on the precipitation of apoliprotein (LDL) by phosphotungestic acid / $MgCl_2$, then centrifuge to sediminate the precipitation and enzymatic analysis of the HDL as residual cholesterol in the supernatant.

Reagent composition

R1: Precipitation reagent / Phosphotungestic acid 0.63 mmol / L, magnesium cholride 25 mmol / L.

R2: Cholesterol MR.

Cal: Cholesterol standard / Cholesterol 50 mg/ dL.

Assay

Put the reagents and samples to room temperature, 0.2 mL of sample or standard was added into the tube containing 0.4 mL of R1 reagent, mix well by vortix for 10 minutes at 25 °C, centrifuge the tube for 5 minutes at 5000 rpm, separate off the supernatant within 2 hours. Admix 1 mL into the R1 reagent tube of each tube labelled blank, sample supernat and standard supernat also add 50 μL of sample supernat into the supernat tube, 50 μL of standard supernat was added into the standard tube, and mix well for 5 minutes at 37 °C and finally read the absurbance at 500 nm. For calculations HDL concentration from the blood sample, see Equation (3.9).

$$\text{HDL cholesterol (mg/ dL)} = (A_{\text{supernatant}} / A_{\text{standard}}) \times C_{\text{standard}} \quad (3.9)$$

3.2.4 Low density lipoprotein (LDL) test

Principle

This assay is based on precipitation of low density lipoproteins by polyvinyl sulfate in serum, centrifuge the precipitant and test as residual cholesterol of VLDL and HDL remaining in the supernatant.

Reagent compositions

R1: Precipitation reagent / polyvinyl sulfate 170 g / L.

CAL: LDL cholesterol standard / Cholesterol 50 mg / L.

Assay

Put the reagents and samples at room temperature, adadd to labelled tube 0.2 mL of sample or standard with 0.1 mL of R1 reagent and vortex for 10 minutes at 25 °C, centrifuge for 10 minutes at 2500 rpm then elute an aliquot of supernatant and measured the total cholesterol of the sample by predispose two series of assayed and measured the remaining cholesterol in the supernatant. Place into the R1 tube 1 mL of each blank, sample and standard supernat then admix 50 µL of the sample supernat to the supernat tube, also add 50 µL of the standard supernat into the standard tube then recite the absurbance at 500 nm. For calculations LDL levels from the blood samples, see Equation (3.10) and Equation (3.11).

$$\text{Cholesterol}_{\text{supernat}}\ (\text{mg/ dL}) = (A_{\text{supernatant}} / A_{\text{standard}}) \times C_{\text{standard}} \quad (3.10)$$

$$\text{LDL cholesterol (mg / dL)} = \text{Total cholesterol} - \text{cholesterol}_{\text{supernatant}} \quad (3.11)$$

3.2.5 HbA1c test

This test is included admix 500 µL of lysing reagent into the tubes then put 100 µL of blood sample and 70 µL of hemolysate in to the specific tubes, place the tubes on the rotator for 5 minutes, then decant into a cuvette then recite the absorbance at 420 nm. Put 5 mL of deionized water then add 20 µL of hemolysate, then tuning the machine to 415 nm, then recite the absorbance values for sample, control and standard. For Calculations HbA1c in the blood samples, see Equation (3.12).

$$\text{HbA1c (\%)} = R_{\text{unknown}} / R_{\text{standard}} \times \text{Standard concentration} \quad (3.12)$$

3.2.6 Glucose test

Glucose is converted to D-glucanate by the aiding glucose oxidase with formation hydrogen peroxidase, these calculations are discribed in equation (3.13) and Equation (3.14). A blend of 4- aminoantipyrine and phenol is oxidized by peroxidase and hydrogen peroxide to form quinonemine and H_2O.

$$\text{B- D- Glucose} \xrightarrow{\text{Glucose oxiase}} \text{D- Glucose} + H_2O_2 \quad (3.13)$$

$$\text{4- Aminoantipyrine + Phenol} \xrightarrow{\text{Peroxidase}} \text{Quinonemine} + H_2O \quad (3.14)$$

Assay

Put 1 mL from blood sample, blank and standard into the monoreagent tube, admix 10 µL of blood sample to the sample tube, 10 µL of standard was added to the cal standard tube, incubate the tube for 10 minutes at 25 °C then recite the absorbance at 500 nm against the blank. For the calculations the levels in the blood samples, it was used Equation (3.15)

$$\text{Glucose (mg / dL)} = \text{A sample / A standard} \times \text{C standard} \quad (3.15)$$

3.2.7 C- reactive protein (CRP) test

Reagent compostion

R1: Diluent / Tris buffer 20 mmol / L.

R2: Latex / Latex particles coated with goat anti – human CRP.

CaL: Calibrator / Human serum.

Assay

Prewarm the cuvette of the photometer and the reagent to 37 °C, by using the distilled water fixate the machine at 540 nm. Put 15 µL of sample or calibrator or blank and 1 mL of reagent into a cuvette, mix well and then induct the cuvette into the instrument and read the absurbance after 5 minutes of calibrator or sample addition. Measured the absurbance difference of each point of the calibration curve and choose the values obtained against the CRP concetration of each calibrator alleviation. Reckon the CRP concetration in the sample by applying its absurbance differences (A_5-A_{blank}) in the calibration curve.

3.2.8 Copeptin test

This assay is based on collection of blood samples into a chilled syringe then put it in a chilled lithium heparin container and centrifuge the container at 5 °C for 5 minutes. Plasma samples were taken into the EDTA tubes then measured the copeptin piptide concentration in duplicate in a single batch with the utilize of a mercantile automated immuno fuoresencs assay (BRAHMS CT-pro AVPLIA, Gmbll, Germany).

3.3 Statistical analysis

Data analysis were accomplish by using SPSS software version 22.0 for windows (IPM, Chicago, IL, USA). T- test and independents samples test were utilized to comparing the means of values, standard deviation and standard error mean besides T- test for equality of means of the tested parameters of copeptin and the other tests at P values ≤ 0.05 was considered as statistically significant.

4. RESULTS AND DISCUSSION

4.1 The Age Mean of the Tested Groups

The aim of the current medical examination is to study the role of copeptin peptide in changes secondary hypogonadism in men with and without type1 and type2 of diabetes mellitus.

The results of Table 4.1 revealed that the mean of age of the current study in DM1 group was 31.02 year, DM2 group was 42.50 year and in control group recorded 44.42 year and the total of age mean was recorded 39.31 year respectivelly.

The stanard deviation of the three tested groups showed that the DM1 group recorded 13.03, DM2 was 8.10 and control group recorded 11.27 (Table 4.1).

Table 4.1 The age mean of the tested groups

Study groups	Mean of age	N	Std. Deviation
DM1	31.0250	40	13.03346
DM2	42.5000	40	8.10191
Control	44.4250	40	11.27713
Total	39.3167	120	12.41576

4.2 Copeptin and its Role in This Study

The results ulustrated in Table 4.2 and Figure 4.1 showed that the mean of copeptin concentration in blood sample of the three tested groups were varied in their values of copeptin, the DM2 group was revealed excel significant on the other groups which recorded 25.83 pg/mL as compared to DM1 and control groups which recorded 16.16 and 5.07 pg/mL respectivelly (Table 4.2).

The standard deviation of the three groups recorded 3.26, 3.79, and 3.58 respectivelly. The elevation of copeptine levels in blood serum a alternate marker of arginine vasopressin and this due to increased the hazard of deabetes disease, the copeptin as a biomarker seems to reflect the disease severity in diabetes patients.

Enhorning *et al.* (2013) showed that the arizing copeptin predicts increment risk for diabetes patients separate of established clinical dangers factors, containing insulin and fasting glucose.

Also Refardt *et al.* (2019) pointed the role of copeptin as a prognostic marker in the implementation of the diabetes insipidus disease after pitutary operation.

Our findings is compatable with the findings of Enhorning *et al.* (2019) which indicated that the maximal increase of copeptin levels were reached to 11.1 ± 4.6 pmol/ L while copeptin levels in diabetes insipidus ptients remined low upon hyperglycemia at 3.7 ± 0.7 pmol/ L.

Katan *et al.* (2007) proved that copeptin measured through hypoglycemia is a beneficial measure to identify diabetes patients.

Table 4.2 Copeptin mean of the study groups

Study groups	Mean	N	Std. Deviation
DM1	16.1625	40	3.26423
DM2	25.8375	40	3.79147
Control	5.0725	40	3.58658
Total	15.6908	120	9.21951

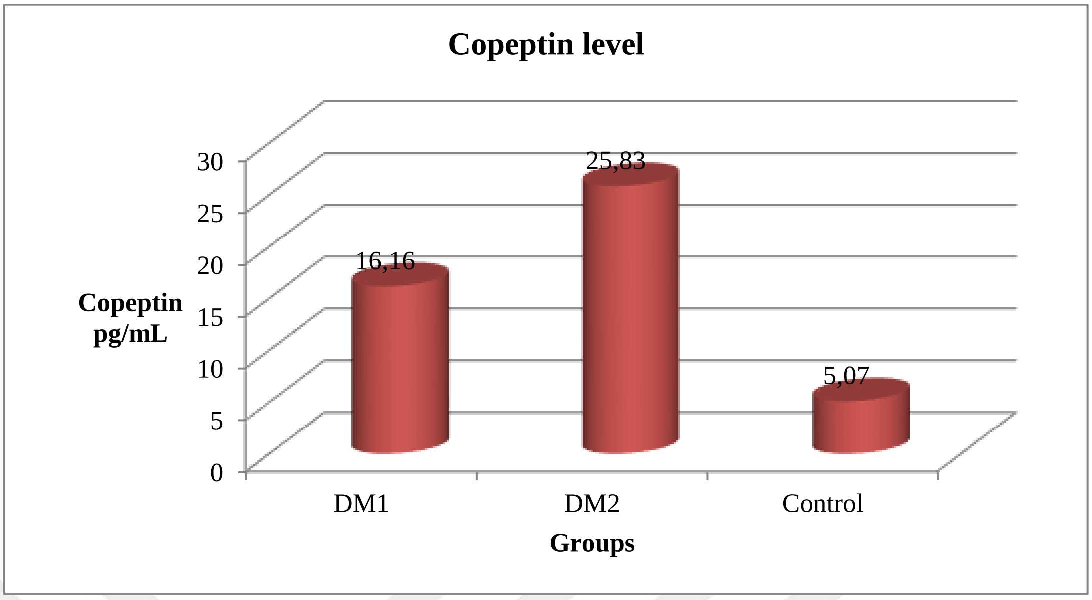

Figure 4.1 Copeptin mean of the study groups

4.3 Copeptin and LDL

The results of the statistics of copeptin and LDL parameters in the three tested groups (DM1, DM2, and Control) showed that DM2 group was excel significant in copeptin and LDL concentrations as compared to the other groups (DM1 and control) which recorded 25.83 pg/ mL and 86.25 mg / dL as compared to DM1 and control groups which recorded (16.16, 5.07) pg/ mL and (83.22, 102.37) mg/ dL respectivelly (Table 4.3 and Figure 4.2).

Table 4.3 Statistics of copeptin and LDL parameters

Study groups		**Copeptin**	**LDL**
DM1	Mean	16.1625	83.2250
	N	40	40
	Std. Deviation	3.26423	13.45932
DM2	Mean	25.8375	86.2500
	N	40	40
	Std. Deviation	3.79147	6.82285
Control	Mean	5.0725	102.3750
	N	40	40
	Std. Deviation	3.58658	3.27921
Total	Mean	15.6908	90.6167
	N	120	120
	Std. Deviation	9.21951	12.22285

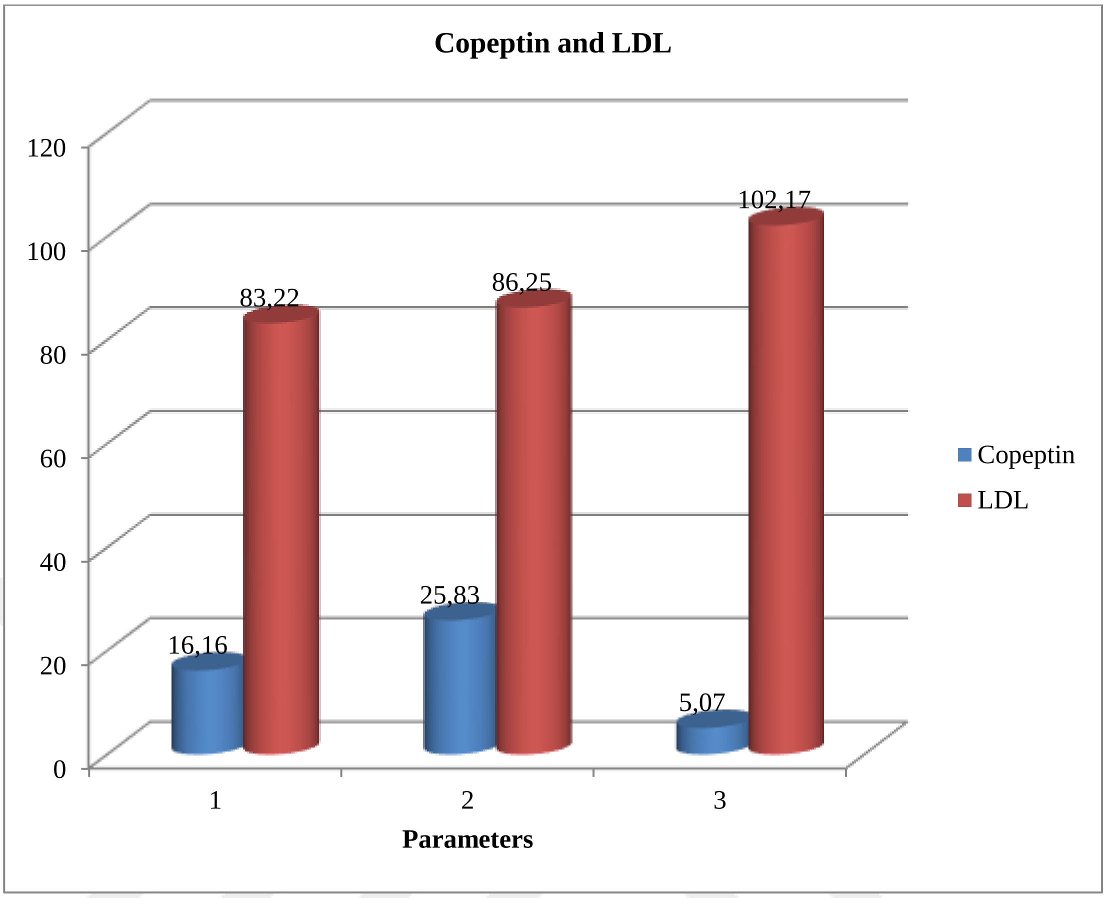

Figure 4.2 Statistics of copeptin and LDL parameters

The Pearson correlations between copeptin and LDL parameters showed it is a significant at 0.01 level (2- tailed) with a negative moderate correlation between them (-0.513), these results are clearly shown in Table 4.4.

Table 4.4 The Pearson correlations between copeptin and LDL parameters

Correlation between copeptin and LDL			
		Copeptin	LDL
Copeptin	Pearson Correlation	1	-.513**
	Sig. (2-tailed)		.000
	N	120	120
LDL	Pearson Correlation	-.513**	1
	Sig. (2-tailed)	.000	
	N	120	120
** Correlation is significant at the 0.01 level (2-tailed).			

4.4 Copeptin and HDL

The results of Table 4.5 and Figure 4.3 showed that the DM2 group was significantly excel on the other groups in HDL concentrations in serum blood, the DM2 recorded 25.83 mg / dL while the DM1 and control groups recorded 16.16 and 5.07 mg / dL respectively.

Table 4.5 Statistics of Copeptin and HDL parameters

Study groups		**Copeptin**	**HDL**
DM1	Mean	16.1625	51.4250
	N	40	40
	Std. Deviation	3.26423	9.30643
DM2	Mean	25.8375	61.4000
	N	40	40
	Std. Deviation	3.79147	9.51059
Control	Mean	5.0725	52.9250
	N	40	40
	Std. Deviation	3.58658	2.95598
Total	Mean	15.6908	55.2500
	N	120	120
	Std. Deviation	9.21951	8.96328

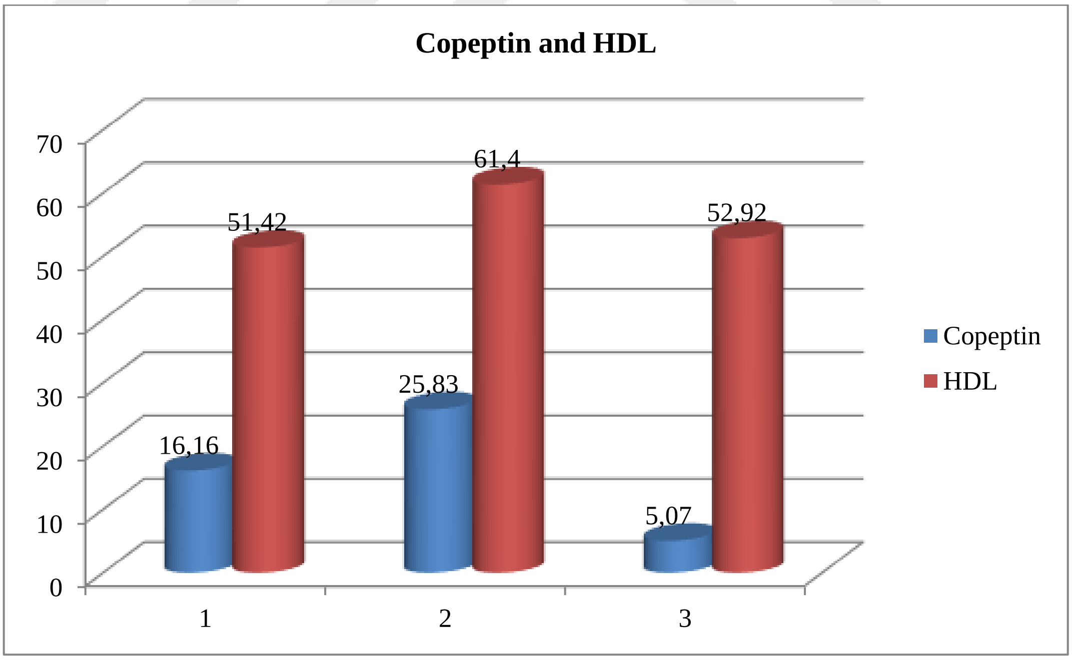

Figure 4.3 Statistics of copeptin and HDL parameters

4.5 Copeptin and Total Triglyceride

The results of Table 4.6 and Figure 4.4 revealed that the mean of triglyceride levels in serum of DM2 patients were significant higher than in DM1 and control groups, the DM2 recorded 185.95 mg/ mL as compared to DM1 and control groups which recorded 168.70 and 119.15 mg / mL respectively.

We found that the elevated of copeptin level associated with the increasing in lipid profile such as total triglycerides, total cholesterol HDL and LDL in diabetes patients.

These findings were in agreement with the findings of Atere *et al.* (2020) they indicated that the level of copeptin and total triglycerol, total cholesterol, HDL, and LDL were significantly higher in both obesed and non-obesed diabetes patients than in control group.

Also Al-Obaidi and Alaaraji (2021) found that the levels of copeptin were higher in DI patients and the concentration of total glycerides, total cholesterol, and LDL were higher as compared to control group.

Tenderenda-Banasink *et al.* (2014) concluded that the high levels of copeptin were associated with the elevated concentration of triglycerides, albumin, uria, and uric acid.

Table 4.6 Statistics of copeptin and triglyceride parameters

Study groups		**Triglyceride**	**Copeptin**
DM1	Mean	168.7000	16.1625
	N	40	40
	Std. Deviation	11.68650	3.26423
DM2	Mean	185.9500	25.8375
	N	40	40
	Std. Deviation	13.37995	3.79147
Control	Mean	119.1500	5.0725
	N	40	40
	Std. Deviation	20.07237	3.58658
Total	Mean	157.9333	15.6908
	N	120	120
	Std. Deviation	32.30910	9.21951

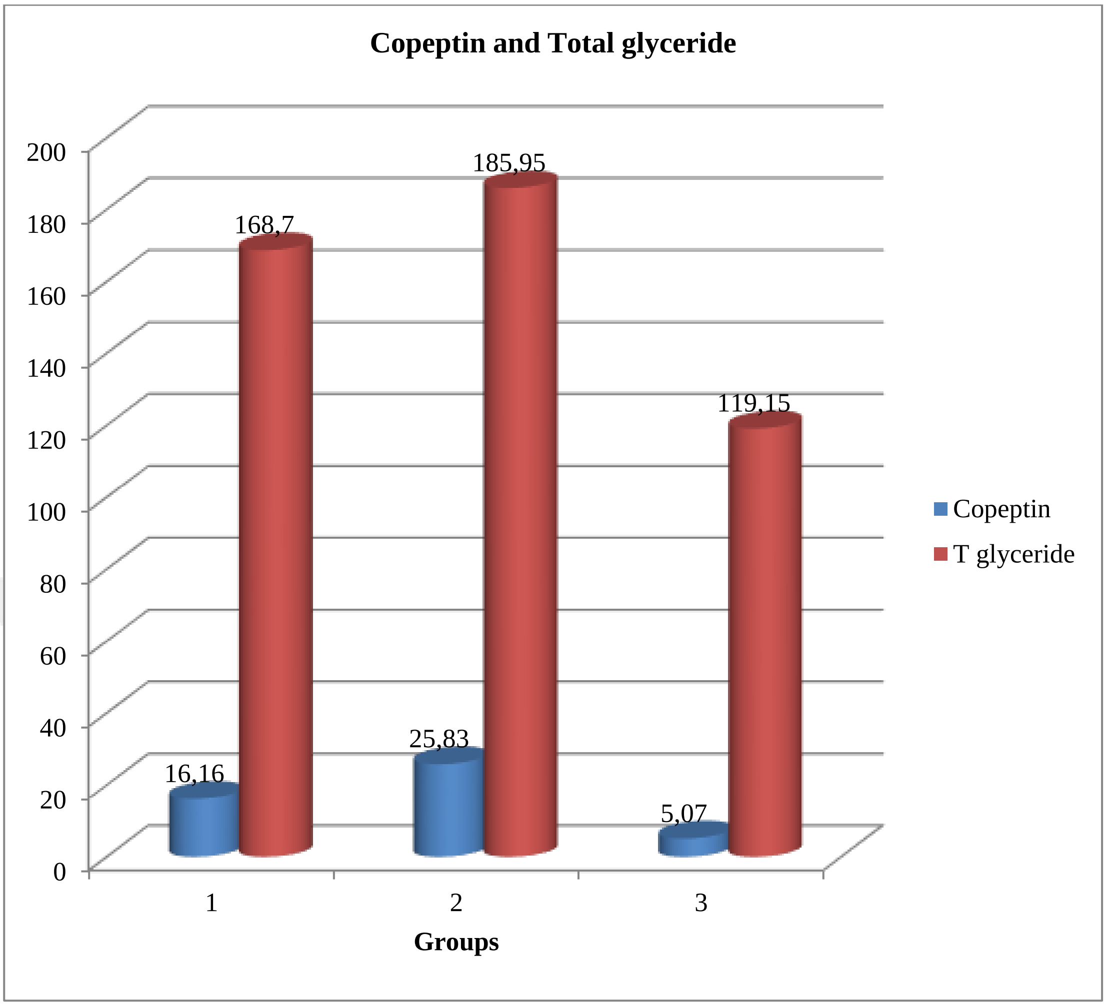

Figure 4.4 Statistics of copeptin and triglyceride parameters

4.6 Copeptin and Total Cholesterol

The results of Table 4.7 showed that the mean of total cholesterol levels in serum of DM2 patients were significant higher than in DM1 and control groups, the DM2 recorded 249.25 mg/ mL as compared to DM1 and control groups which recorded 235.87 and 160.97 mg / mL respectively.

The levels of total cholesterol were associated with the copetine level in both of DM1 and DM2 patients as compared to healthy individuals, which considers as a biomarker for diabetes disease.

Table 4.7 Statistics of copeptin and total cholesterol parameters

Study groups		**Copeptin**	**Cholesterol**
DM1	Mean	16.1625	235.8750
	N	40	40
	Std. Deviation	3.26423	24.90720
DM2	Mean	25.8375	249.2500
	N	40	40
	Std. Deviation	3.79147	36.35985
Control	Mean	5.0725	160.9750
	N	40	40
	Std. Deviation	3.58658	18.31139
Total	Mean	15.6908	215.3667
	N	120	120
	Std. Deviation	9.21951	47.62563

The Pearson correlations between copeptin and total cholesterol parameters showed it is a significant at 0.01 level (2- tailed) with a positive high correlation between them (0.754), the elevated in total cholesterol level was associated with increasing in total cholesterol levels considers as a biomarker of diabetes mellitus disease (Table 4.8 and Figure 4.5).

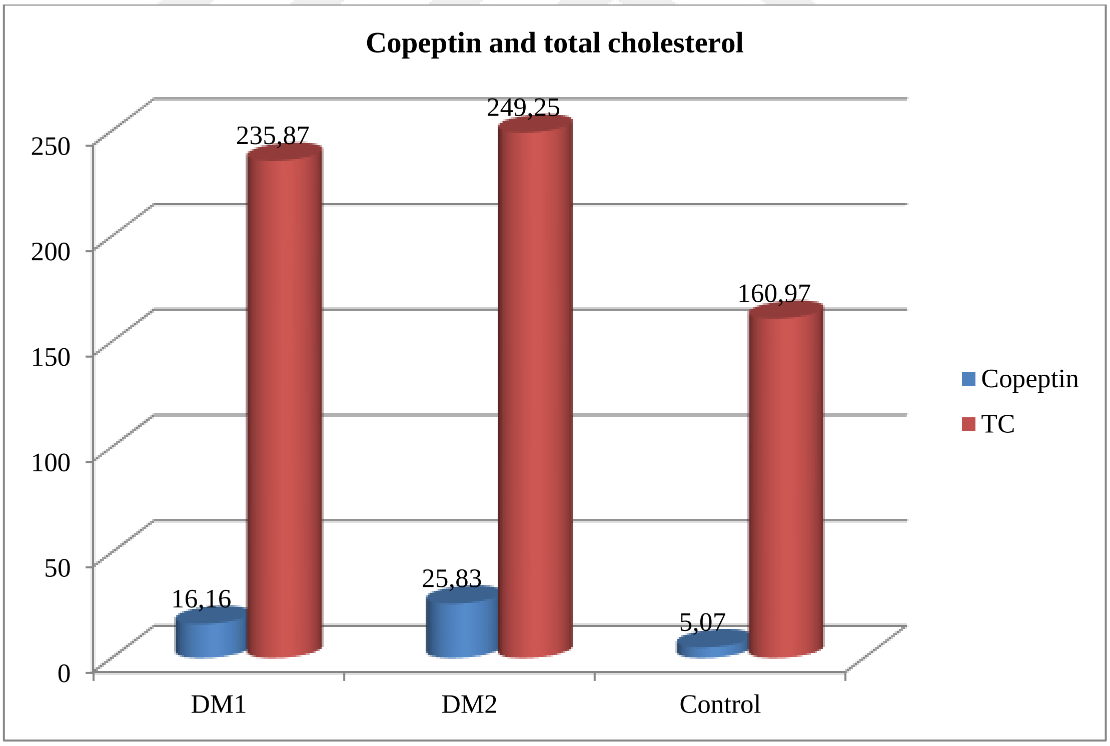

Figure 4.5 Statistics of copeptin and total cholesterol parameters

Table 4.8 The Pearson correlations between copeptin and total cholesterol parameters

		Copeptin	Cholesterol
Copeptin	Pearson Correlation	1	.754**
	Sig. (2-tailed)		.000
	N	120	120
Cholesterol	Pearson Correlation	.754**	1
	Sig. (2-tailed)	.000	
	N	120	120
**. Correlation is significant at the 0.01 level (2-tailed)			

4.7 Copeptin and glucose concentration

The results of Table 4.9 and Figure 4.6 indicated that the mean of glucose levels in serum of DM2 patients were significant higher than in DM1 and control groups, the DM2 recorded 171.05 mg/ mL as compared to DM1 and control groups which recorded 136.22 and 83.17 mg / mL respectively.

These findings were in agreement with the findings of Al-Bayati *et al.* (2021) which they showed that the copeptin level was significantly higher in obese group as compared with control group and there was a correlation between copeptin and blood sugar levels.

Table 4.9 Statistics of copeptin and glucose parameters

Study groups		Copeptin	Glucose
DM1	Mean	16.1625	136.2250
	N	40	40
	Std. Deviation	3.26423	7.08732
DM2	Mean	25.8375	171.0500
	N	40	40
	Std. Deviation	3.79147	11.66839
Control	Mean	5.0725	83.1750
	N	40	40
	Std. Deviation	3.58658	16.43618
Total	Mean	15.6908	130.1500
	N	120	120
	Std. Deviation	9.21951	38.28896

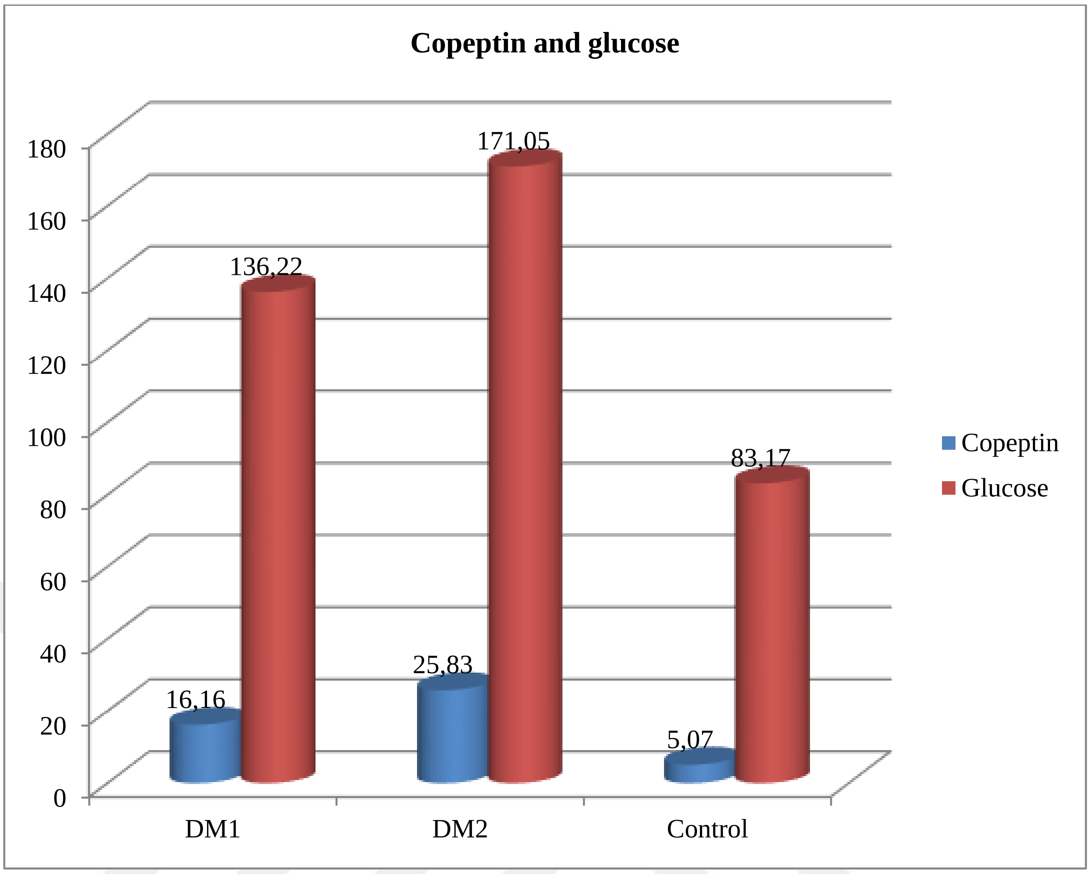

Figure 4.6 Statistics of copeptin and glucose parameters

The Pearson correlations between copeptin and mean of glucose in blood showed it is a significant at 0.01 level (2- tailed) with a strong positive correlation between them (0.928), the elevated in total cholesterol level was associated with increasing in glucose levels considers as a biomarker of diabetes mellitus disease (Table 4.10).

Table 4.10 The Pearson correlations between copeptin and total glucose parameters

		Copeptin	Glucose
Copeptin	Pearson Correlation	1	.928**
	Sig. (2-tailed)		.000
	N	120	120
Glucose	Pearson Correlation	.928**	1
	Sig. (2-tailed)	.000	
	N	120	120
**. Correlation is significant at the 0.01 level (2-tailed).			

4.8 Copeptin and HbA1C

The results of Table 4.11 pointed that the mean of HbA1C levels in serum of DM2 and DM1 patients were significant higher than in healthy persons, which recorded 9.30 and 8.06 % as compared to control group which recorded 5.33 % respectively.

These results were in agreement with the results of Then *et al.* (2015) which showed that copeptin has been labeled as biomarker for diabetes type 2 and they pointed that elevated in copeptin levels were linked with diabetes type 2 and HbA1C in men patients only.

Also, Velho *et al.* (2013) showed that the high levels of copeptin were strongly linked with the risk of severe renal findings in diabetes type 2 patients, this link was independent of age of patient ,blood pressure and HbA1C.

Table 4.11 Statistics of Copeptin and HbA1C parameters

Study groups		**Copeptin**	**HbA1C**
DM1	Mean	16.1625	8.0625
	N	40	40
	Std. Deviation	3.26423	.35855
DM2	Mean	25.8375	9.3075
	N	40	40
	Std. Deviation	3.79147	1.35351
Control	Mean	5.0725	5.3350
	N	40	40
	Std. Deviation	3.58658	1.22632
Total	Mean	15.6908	7.5683
	N	120	120
	Std. Deviation	9.21951	1.97756

The Pearson correlations between copeptin and mean of HbA1C in blood showed it is a significant at 0.01 level (2- tailed) with a strong positive correlation between them (0.783), the elevated in HbA1C level was associated with increasing in copeptin levels considers as a biomarker of diabetes mellitus disease (Table 4.12 and Figure 4.7).

Table 4.12 The Pearson correlations between copeptin and HbA1C parameters

		Copeptin	HbA1C
Copeptin	Pearson Correlation	1	0.783**
	Sig. (2-tailed)		0.000
	N	120	120
HbA1C	Pearson Correlation	0.783**	1
	Sig. (2-tailed)	0.000	
	N	120	120
** Correlation is significant at the 0.01 level (2-tailed)			

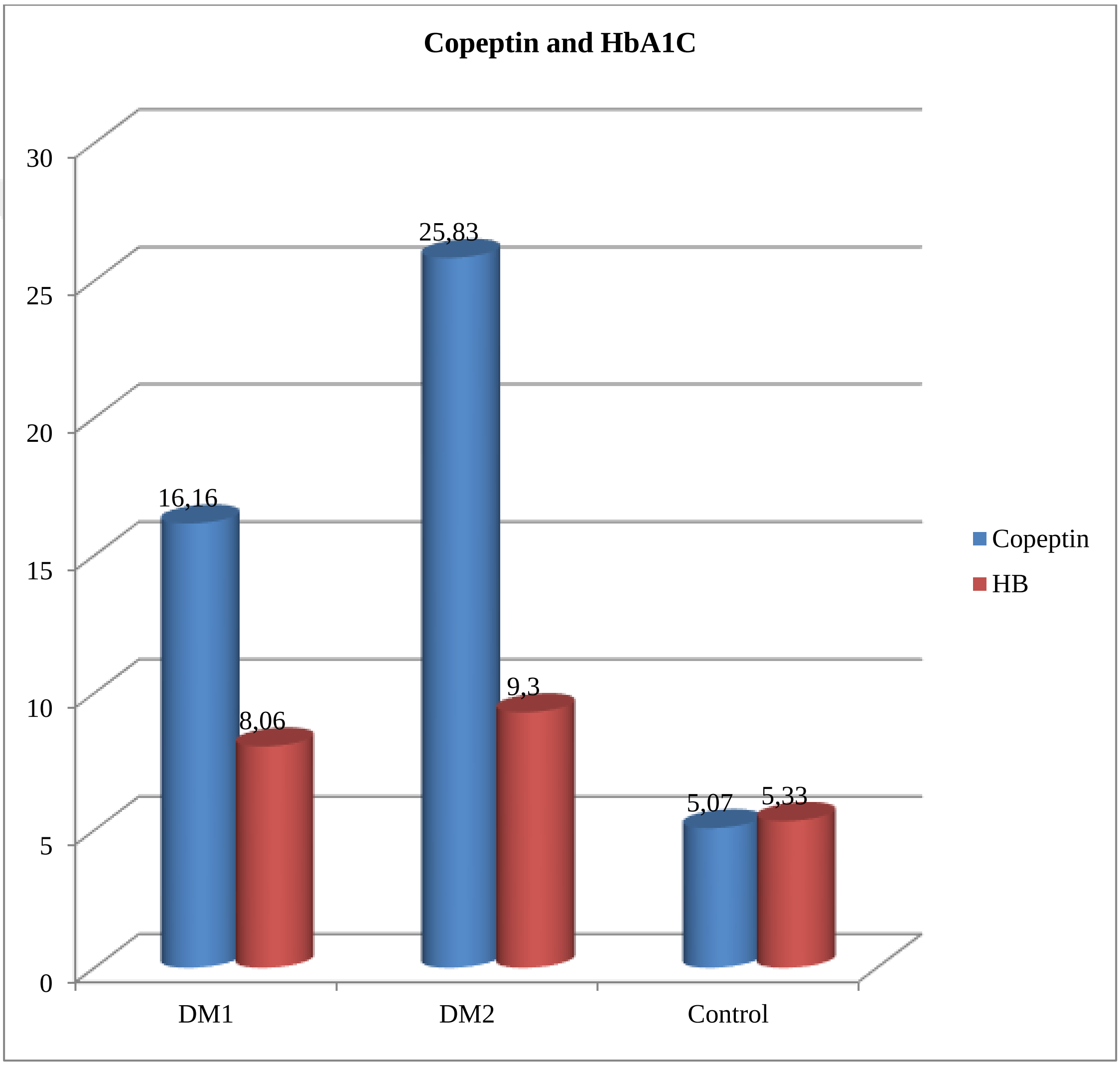

Figure 4.7 The Pearson correlations between copeptin and HbA1C parameters

4.9 Copeptin and CRP

The results of Table 4.13 revealed that the mean of CRP levels in serum of DM2 and DM1 patients were significant higher than in healthy persons, which recorded 12.34 pg/mL and 10.44 pg/mL as compared to control group which recorded 4.72 pg/mL respectively.

Abbasi *et al.* (2012) revealed that plasma copeptin level a lone or associated with CRP and glucose was significantly ameliorated the hazard prediction for diabetes in women patients.

Gomaa and Muhammed (2019) showed that the elevated copeptin was correlated with CRP levels in metabolic syndrome.

Table 4.13 Statistics of Copeptin and CRP parameters

Study groups		Copeptin	CRP
DM1	Mean	16.1625	10.4450
	N	40	40
	Std. Deviation	3.26423	0.84184
DM2	Mean	25.8375	12.3400
	N	40	40
	Std. Deviation	3.79147	1.22009
Control	Mean	5.0725	4.7275
	N	40	40
	Std. Deviation	3.58658	1.85195
Total	Mean	15.6908	9.1708
	N	120	120
	Std. Deviation	9.21951	3.52168

The Pearson correlations between copeptin and mean of CRP in blood showed it is a significant at 0.01 level (2- tailed) with a strong positive correlation between them (0.829), the elevated in CRP level was linked with increasing in copeptin levels considers as a biomarker of diabetes mellitus disease (Table 4.14 and Figure 4.8).

Table 4.14 The Pearson correlations between copeptin and CRP parameters

		Copeptin	CRP
Copeptin	Pearson Correlation	1	.829**
	Sig. (2-tailed)		.000
	N	120	120
CRP	Pearson Correlation	.829**	1
	Sig. (2-tailed)	.000	
	N	120	120
**. Correlation is significant at the 0.01 level (2-tailed).			

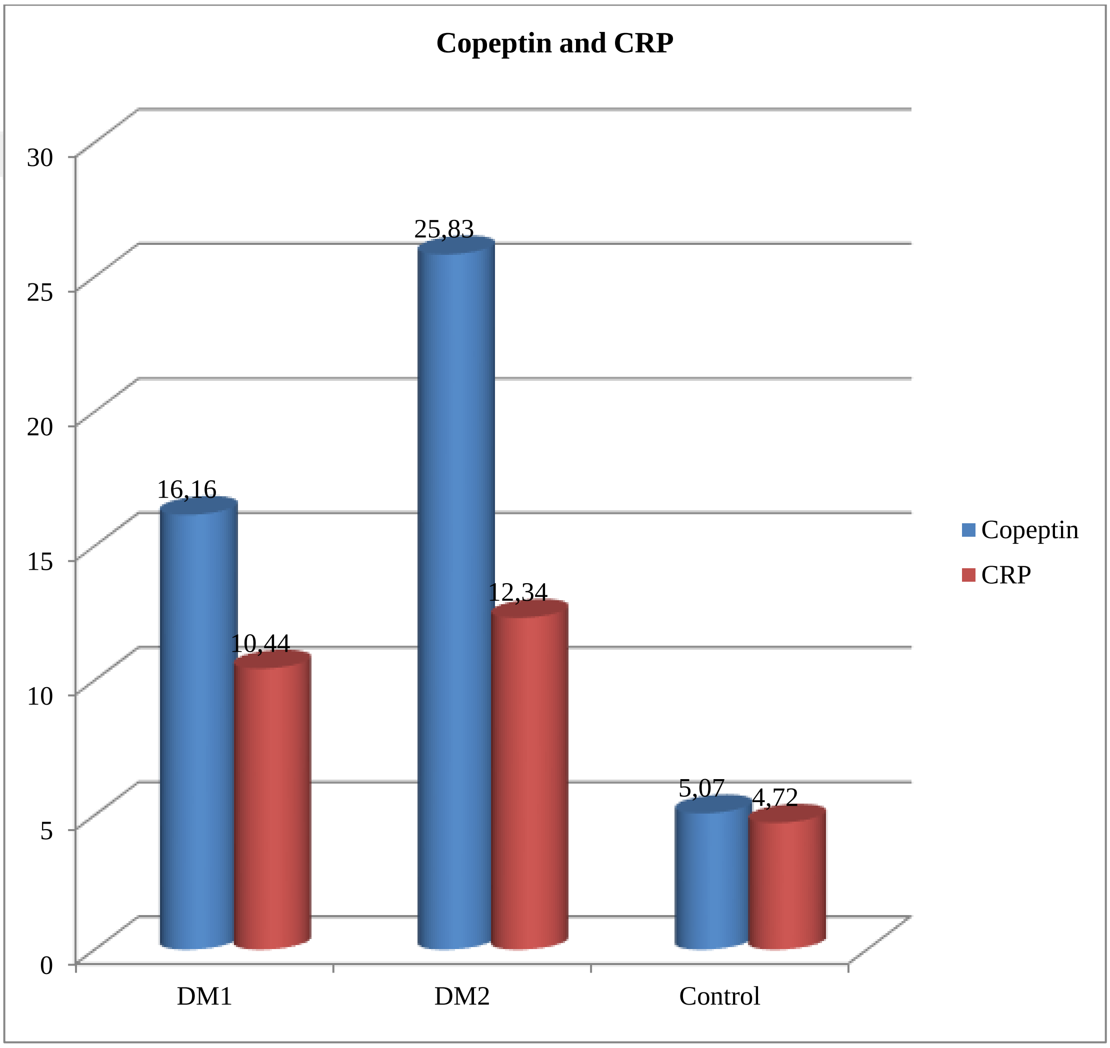

Figure 4.8 The Pearson correlations between copeptin and CRP parameters

5. CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

The results showed that the mean of copeptin concentration in blood sample of the three tested groups were varied in their values of copeptin, the DM2 group was revealed excel significant on the other groups which recorded 25.83 pg/mL as compared to DM1 and control groups which recorded 16.16 and 5.07 pg/mL respectivelly.

The copeptin as a biomarker seems to reflect the disease severity in diabetes patients.

We found that the elevated of copeptin level associated with the increasing in lipid profile such as total triglycerides, total cholesterol HDL and LDL in diabetes patients which considers as a biomarker for diabetes disease.

The results of copeptin and LDL parameters in the three tested groups (DM1, DM2, and Control) showed that DM2 group was excel significant in copeptin and LDL concentrations as compared to the other groups (DM1 and control).

The results pointed that the mean of HbA1C levels in serum of DM2 and DM1 patients were significant higher than in healthy persons, which recorded 9.30 and 8.06 % as compared to control group which recorded 5.33 % respectively, the elevated in HbA1C level was associated with increasing in copeptin levels considers as a biomarker of diabetes mellitus disease.

The results also revealed that the mean of CRP levels in serum of DM2 and DM1 patients were significant higher than in healthy persons, which recorded 12.34 pg/mL and 10.44 pg/mL as compared to control group which recorded 4.72 pg/mL respectively.

5.2 Recommendations

Study the efficiency of copeptin peptide as a biomarker of another chronic diseases and identify their role in human physiology.

Research of another peptides or hormones may be played beneficial roles in early detection of chronic diseases. The early detection of these diseases may be assists in medicated theses illnesses.

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