Effect of Ketogenic Diet on Serum IL10, MDA, Nitrate, and other Biochemical Parameters in Obese Individuals

A Thesis
Submitted to the Council of the Medical Laboratory Technology Department, Erbil Health and Medical Technical College, Erbil Polytechnic University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Medical Laboratory Technology

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وَقُلِ اعۡمَلُواْ فَسَيَرَى اللَّهُ
عَمَلَكُمۡ
وَرَسُولُهُۥ
وَٱلۡمُؤۡمِنُونَۖ
وَ سَتُرَدُّونَ إِلَىٰ عَٰلِمِ
ٱلۡغَيۡبِ
وَالشَّهَٰدَةِ فَيُنَبِّئُكُم بِمَا كُنتُمۡ
تَعۡمَلُونَ

DECLARATION

I declare that the Master Thesis entitled: **Effect of Ketogenic Diet on Serum IL10, MDA, Nitrate ,and other Biochemical Parameters in Obese Individuals** is my own original work, and hereby certify that unless stated, all work contained within this thesis is my own independent research and has not been submitted for the award of any other degree at any institution, except where due acknowledgment is made in the text.

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This thesis has been written under my supervision and has been submitted for the award of the degree of Master of MLT with my approval as supervisor.

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Head of the MLT Department

Date**: / / 2022**

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We certify that we have read this thesis: Effect of Ketogenic Diet on Serum IL10, MDA, Nitrate, and other Biochemical Parameters in Obese Individuals and as an examining committee examined the student (Ahmad Abdulrazzaq Bapir) in its content and what related to it. We approve that it meets the standards of a thesis for the degree of MSc. in MLT (Biochemistry).

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Dedication

This thesis is dedicated to:

My mother and memory of my father…

My always encouraging, ever faithful brothers and sisters…

Ahmad Abdulrazzaq Bapir

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SUMMARY

The ketogenic diet (KD) shifts body metabolism away from carbohydrates toward fat and ketone bodies. Many overweight individuals suffer from insulin resistance and other symptoms of prediabetes in the world. Thus, this study aims to investigate the safety of KD and its adverse actions on prediabetic overweight and obese individuals. In this case-control study, fifty-eight overweight and obese individuals were divided into prediabetes and control groups based on their Homeostasis Model Assessment-estimated Insulin Resistance (HOMA-IR) values. Both groups performed KD for 3 months. During this period, blood ketone bodies were monitored weekly, and Fasting blood Sugar (FBS), lipid profile, Creatinine, urea, uric acid, Blood nitrogen urea (BUN), Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), C peptide level, oxidative stress, and interleukin 10 (IL10) were analysed before and after the intervention. BMI and FBS reduced significantly after the KD for both groups. However, both groups increased significantly triglyceride (TG), Creatinine, urea, uric acid, and BUN (Blood nitrogen urea). While the cholesterol and interleukin 10 (IL10) were elevated in the control group. In the other hand HDL (High density lipoprotein), NO (nitric oxide) and MDA (Malondialdehyde) were significantly rised but LDL (Lower density lipoprotein) was reduced in prediabetes group after KD.as well C peptide increased significantly in prediabetes group. Also, KD has a significant effect on HOMA-IR and HOMA-S% on both groups but HOMA-B% shows a significant effect only in prediabetes group.

The study reveals that KD is helpful for weight reduction, but also it has a positive effect on reducing insulin resistance in prediabetic individuals.

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List of Abbreviations

The following table describes the significance of various abbreviations used throughout the thesis. The abbreviations that are used for one time are not mentioned in this list.

Abbreviations	Meaning
%	Percent
4-AAP	4-amino-antipyrine
4NPP	4-nitrophenylphosphate ester
Ab	Antibody
AD	Alzheimer's disease
AGEs	advanced glycation end products
AHA	American Heart Association
ALP	alkaline phosphatase
AMP	2-Amino-2-methyl-1-propanol
ATP	Adenosine triphosphate
BHB	Beta-hydroxybutyrate
BMI	Body Mass Index
BUN	Blood urea nitrogen
cal	International Calorie
CE	Cholesterol esterase
CHOP	Cholesterol oxidase
cm	Centimetre
CRP	C-reactive protein
CSF	Cerebrospinal fluid
DA	Dalton
dL	Deciliter
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assays
FBS	Fasting Blood Sugar

FSH	follicle-stimulating hormone
g	Gram
G-6-P	Glucose 6-phosphate
G6PDH	Glucose-6-Phosphate Dehydrogenase
GABA	Gamma-aminobutyric acid
GI	Glycemic index
GLDH	Glutamate dehydrogenase
GLUT1-DS	Glucose transporter 1 deficiency syndrome
GPO	Glycerol Phosphate Oxidase
Hb	Haemoglobin
HbA1c	Hemoglobin A1C
HDL	High-Density Lipoprotein
HK	Hexokinase
HMG-CoA	β-Hydroxy β-methylglutaryl-CoA
HOMA B%	Homeostatic model assessment for beta cell function
HOMA IR	Homeostatic model assessment for insulin resistance
HOMA S%	Homeostatic model assessment cell sensitivity to
HPLC	High Performance Liquid Chromatography
HRP	horseradish peroxidase
IAA	insulin autoantibodies
ICA	islet cell antibodies
IDF	International Diabetes Federation
IFCC	The International Federation of Clinical Chemistry and Laboratory Medicine
IFCC	International Federation of Clinical Chemistry
IGF-1	insulin-like growth factor binding protein 1
IL10	Interleukin 10
IL-6	lnterleukin-6
KATP channels	ATP-sensitive potassium channels
KD	Ketogenic Diet

KFT	Kidney function test
L	Litre
LDH	Lactate dehydrogenase
LDL	Low-Density Lipoprotein
LFT	Liver function test
LGID	Low Glycemic Index Diet
LH	luteinizing hormone
LPL	Lipoprotein lipase
MAD	Modified Atkins Diet
MCT	Medium Chain Triglycerides
MCT1	monocarboxylate transporter 1
MDA	Malondialdehyde
MDH	Malate Dehydrogenase
mmol	Millimole
MPTP	1methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NAD	Nicotinamide adenine dinucleotide
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
NCEP	National Cholesterol Education Program
NF- κ B	Nuclear factor kappa-light-chain-enhancer of activated B cells
NHLBI	National Heart, Lung and Blood Institute
NNED	N-naphthyl-ethylenediamine
NO	Nitric oxide
PCOS	Polycystic ovary syndrome
PD	Parkinson's disease
PEG	Polyethylene Glycol
POD	Peroxidase
PPAR γ	Peroxisome proliferator-activated receptor gamma
ROS	Reactive oxygen species
RPM	Round per minute

T2DM	Type 2 diabetes mellitus
TCA	Trichloroacetic acid
TCA Cycle	Krebs or citric acid cycle
TG	Triglycerides
TNF	Tumor necrosis factor
TNF-α	Tumor necrosis factor alpha
VGLUT2	Vesicular glutamate transporters 2
VLDL	Very Low-Density Lipoprotein
WHO	World Health Organization
β	Beta
γ	Gama

INTRODUCTION

1. INTRODUCTION

Prediabetes is an intermediate stage between normal glycemia and diabetes and is highly prevalent, especially in obese individuals.Risks of major complications in persons with prediabetes, including diabetes, cardiovascular disease, kidney disease, and death. (Echouffo-Tcheugui and Selvin, 2021).

In addition, the increased inflammation levels and an altered incretins response are also under the scope of Prediabetes (Fändriks, 2017). It is estimated that 425 million individuals worldwide have diabetes in 2019, with an additional 374 million experiencing the metabolic state of prediabetes. (Altuve and Severeyn, 2019). Elevated blood glucose levels are a hallmark of Prediabetes. Since prediabetes usually has no noticeable symptoms, it can quickly progress to type 2 diabetes with many-micro-and macro-vascular complications (Ribeiro et al., 2019, Freeman and Pennings, 2021).

The ketogenic diet (KD) is a very high fat, low carbohydrate diet that mimics the metabolic state of fasting (Stafstrom and Rho, 2012) .This puts the body into the metabolic state of ketosis, where ketone bodies are produced to ensure energy. The very high-fat content of KD prompts the production of ketone bodies in the liver from fatty acids from dietary fat. These ketone bodies are used in place of glucose from carbohydrates as the primary source of energy. "Keto-genic diet" thus means a diet in which ketones are produced (Poff et al., 2020). This diet would make it possible to reduce complications of diabetes by activating genes regulating the growth of beta cells in the islets of Langerhans, responsible for insulin production in the pancreas (Goday et al., 2016). Weight loss, a decrease in glucose resistance, and an increase in insulin sensitivity are all benefits associated with low-carbohydrate diets like the KD. (Stocker et al., 2019b). The weight loss by KD leads to an improvement in insulin sensitivity and a reduction in the risk of cardiovascular disease (Reaven, 2005). In addition, the favorable effect of KD in obese patients with polycystic

ovarian syndrome (PCOS) is reported as resulting in the reduction of androgen hormone, regulation of the menstrual cycle, and an increased likelihood of fertility. (Zhang et al., 2018). Studies have shown that the KD can lower inflammatory indicators like C-reactive protein, therefore it may have anti-inflammatory effects (CRP) (Hallberg et al., 2018). The main disadvantages of KD are gastrointestinal discomforts and hypoglycemia that occurs in the first week of KD when the body switches to ketosis (Pascual et al., 2014). Furthermore, the KD has other side effects, including increase serum creatinine ,urea, uric acid, production of kidney stones, hypercholesterolemia, carnitine deficiency, and calcium deficiency (Gautschi et al., 2015, Kossoff et al., 2009). Moreover, atherosclerosis is another potential risk of KD due to increase of level of serum Cholesterol and TG (Fändriks, 2017).

Aims of the study:

The primary aim was to know the safety and Efficacy of KD on weight reduction and insulin resistance in obese prediabetes individuals; the secondary objective was to compare KD in Control and prediabetes individuals.

The objectives of this study were to evaluate the following parameters:

1- Evaluation of FBS and BMI before and after applying KD.
2- Measurement of serum biochemical parameters such as Lipid profile, KFTs (Urea, Uric Acid, creatinine and BUN) and LFTs (ALP, AST, ALT).
3- Measuring Serum C-Peptide and Calculation of HOMA-IR for evaluation of insulin resistance, HOMA-S% for cells sensitivity to insulin and HOMA-B% for beta cell function.
4- Detection of serum MDA and NO as oxidative stress indicators and IL10.

LITERATURE REVIEW AND THEORETICAL BACKGROUND

2. LITERATURE REVIEW AND THEORETICAL BACKGROUND

A ketogenic diet (KD) is a diet that has been used for almost 100 years to treat children with epilepsy; its effectiveness in treating this epilepsy pathology has been demonstrated (Vorin et al., 2003, Bodenant et al., 2008). It made it possible to reduce or eliminate the symptoms linked to the disease when the diet could be followed correctly. This dietary practice for therapeutic purposes was discovered in 1921 by Dr. Russell Wilder, wishing to imitate the effects of fasting (production of ketone bodies) without massive loss of muscle mass. A renewed interest in this method is currently observed, following new research showing its effectiveness on other pathologies. Therefore, this diet can decrease symptoms of several neurological diseases (such as Alzheimer's and Parkinson), T2DM, and Cancer (Steven and Taylor, 2015).

2.1 Ketosis and the ketogenic diet.

The body tends to go into ketosis when there is a fasting situation or low carbohydrate consumption for a prolonged period. Ketosis is a metabolic situation caused by a carbohydrate deficit in which the oxidation of fatty acids is diverted to the production of ketones in the liver (Deemer et al., 2020). There are insufficient glucose stores when entering ketosis; the body looks for other energy sources since glucose is necessary for producing oxaloacetates, an essential intermediate metabolite of various metabolic pathways. In addition, given this shortage of glucose, the central nervous system cannot use fatty acids since they do not cross the blood-brain barrier (Paoli, 2014). The alternative energy source is found in the overproduction of acetyl coenzyme A, which leads to the production of alternative fuel, ketone bodies. The KD is an eating plan based on low carbohydrate intake, high fat intake and moderate protein intake to generate a situation of ketosis in the body similar to that which occurs

when there is a situation of fasting. Therefore, the goal of the KD is for the body to start using ketone bodies, produced from the breakdown of fat, as an energy source instead of using glucose. Therefore, it is a diet based on reducing carbohydrate consumption(Stocker et al., 2019a). In this type of diet, the ratio of fat to carbohydrates and protein varies between 2:1 and 4:1, consisting of 1g/kg of protein, 10-15g of carbohydrates daily, and the rest of the calories consumed being acids, long-chain fats (90% of total energy consumed) (Wheless, 2008). It intends to force the body to use fat as a primary energy source. The foods that usually eaten during KD program are palatable due to their high-fat content. They are usually bacon, eggs, olive oil, fish, poultry, avocado, etc (Stocker et al., 2019a).Three ketone bodies are exist; acetone, acetoacetate, and D-ß-hydroxybutyrate. Acetone is produced in low quantities and exhaled as a volatile compound, producing a characteristic breath odor (Sumithran and Proietto, 2008). The main ketone body produced is acetoacetate, but the primary circulating ketone is D-β-hydroxybutyrate (Paoli et al., 2013a) and both are excreted in the urine. After 3 or 4 days of fasting or following a very low-carbohydrate diet, such as the ketogenic diet, the production of these ketone bodies or ketogenesis will take place in the mitochondrial matrix of the liver since it supplies the necessary energy (Paoli, 2014). Firstly, the formation of acetoacetate occurs when two molecules of acetyl-CoA condense enzymatically in the liver; this reaction is catalyzed by thiolase. Acetoacetyl-CoA and acetyl-CoA then condense to form β-hydroxy-β-methyl glutaryl-CoA (HMG-CoA), which cleaves to form free acetoacetate and acetyl-CoA. This acetolactate can lead to the formation of acetone through the reaction catalyzed by the enzyme acetoacetate decarboxylase (Figure 2.1).

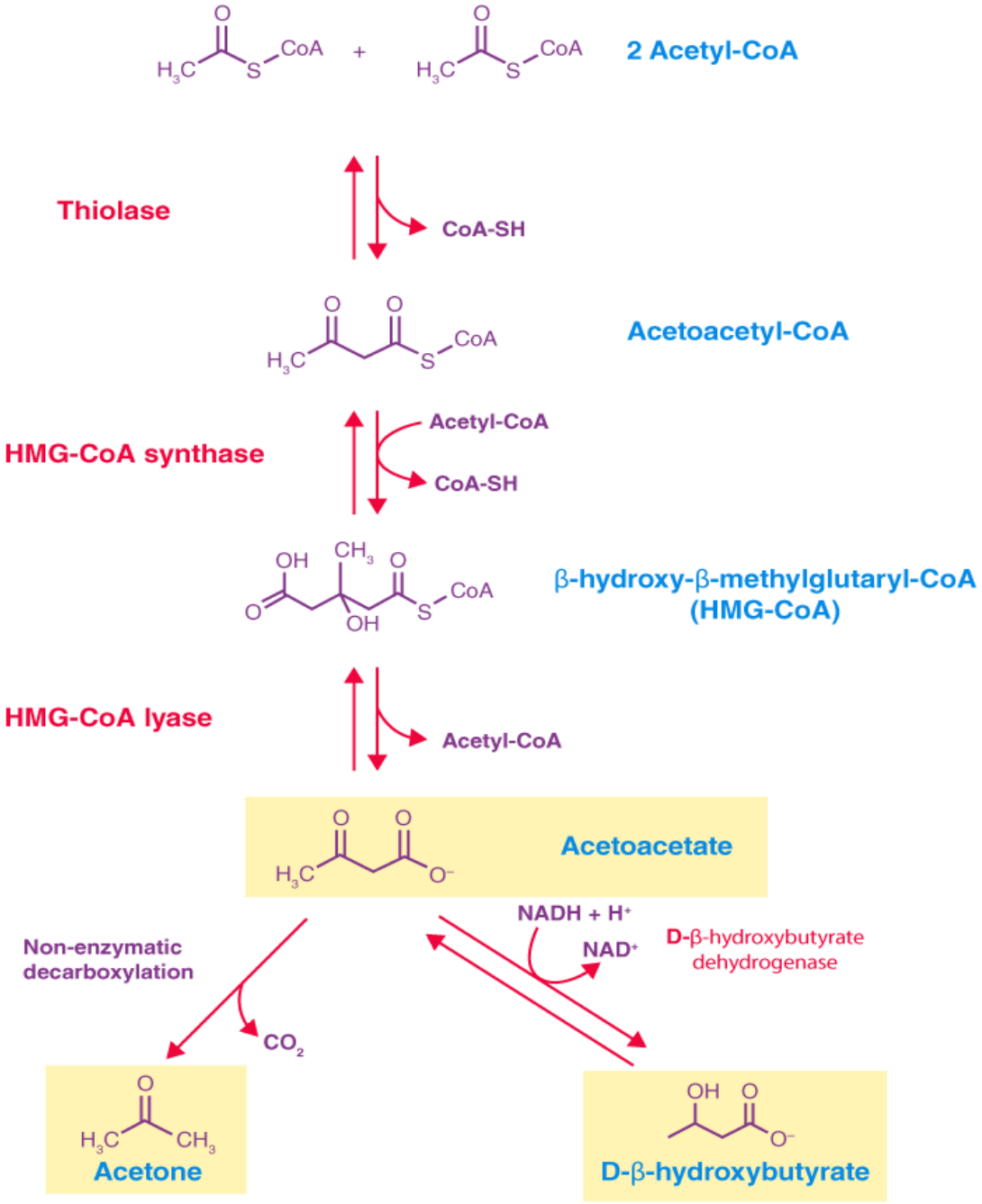


Figure 2.1: The process of ketone bodies formation of ketogenesis (Paoli, 2014)

On the other hand, ketosis is the process by which ketone bodies become acetyl-CoA, which is involved in energy production. This process occurs in the mitochondria of extrahepatic tissues. The D-β-hydroxybutyrate changes to

acetoacetate again, which is then transformed into acetoacetyl-CoA. Finally, two acetyl-CoA molecules are formed that are oxidized in the Krebs cycle for energy production (Paoli, 2014). Under normal conditions, the blood concentration of ketone bodies is less than 0.3 mmol/L. This concentration is deficient compared to glucose (4 mmol/L), so when the concentration of ketone bodies fluctuates around 4 mmol/L, they can be used as a source of energy by the central nervous system (Paoli, 2014). To check that the body has entered ketosis, The level of ketone bodies can be measured through the breath, urine or blood test (Figure 2.2).

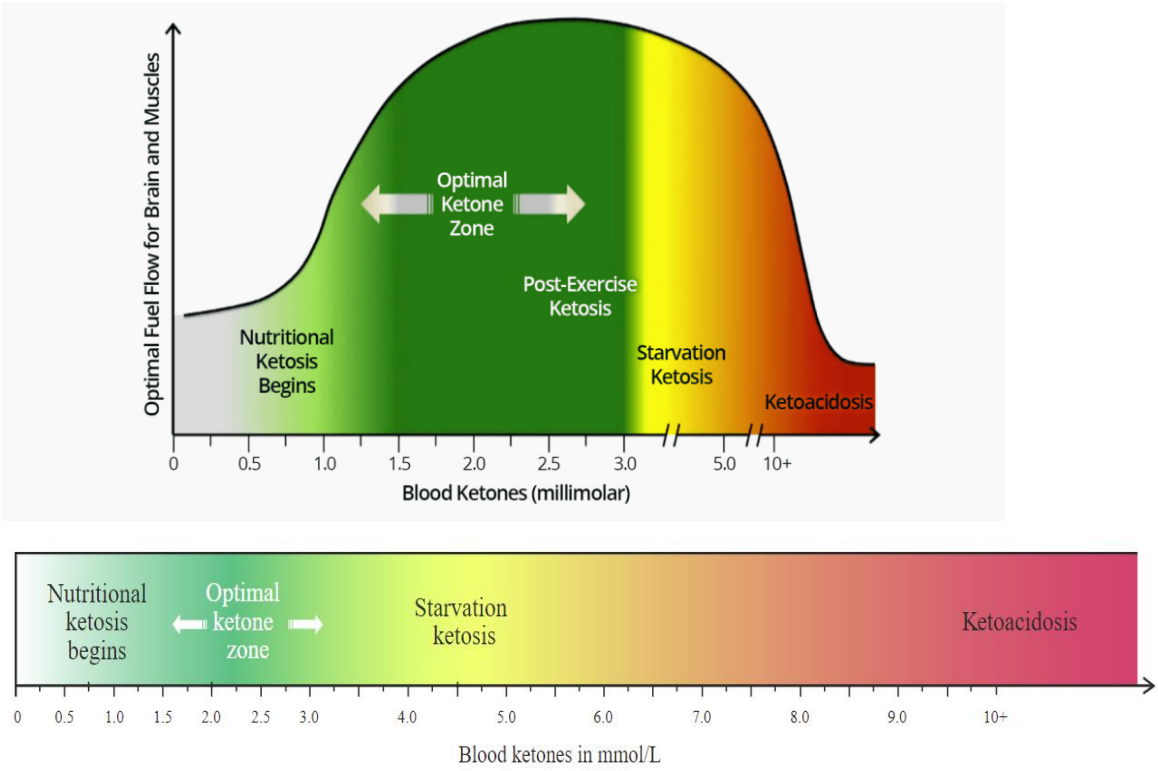


Figure 2.2 Difference in the amount of ketones in the blood between Nutritional ketosis and ketoacidosis(Dearlove et al., 2019)

2.2 Types of the ketogenic diet.

In addition to the classic KD, some variations have been created, such as the KD rich in medium chain triglycerides (MCT), the modified Atkins diet (MAD, of the English Modified Atkins Diet), the low glycemic index diet (LGIT) and Low Glycemic Index Treatment. As shown in figure 2.3, they mainly differ in the percentage of fats, proteins and carbohydrates consumed in each case (Gano et al., 2014).

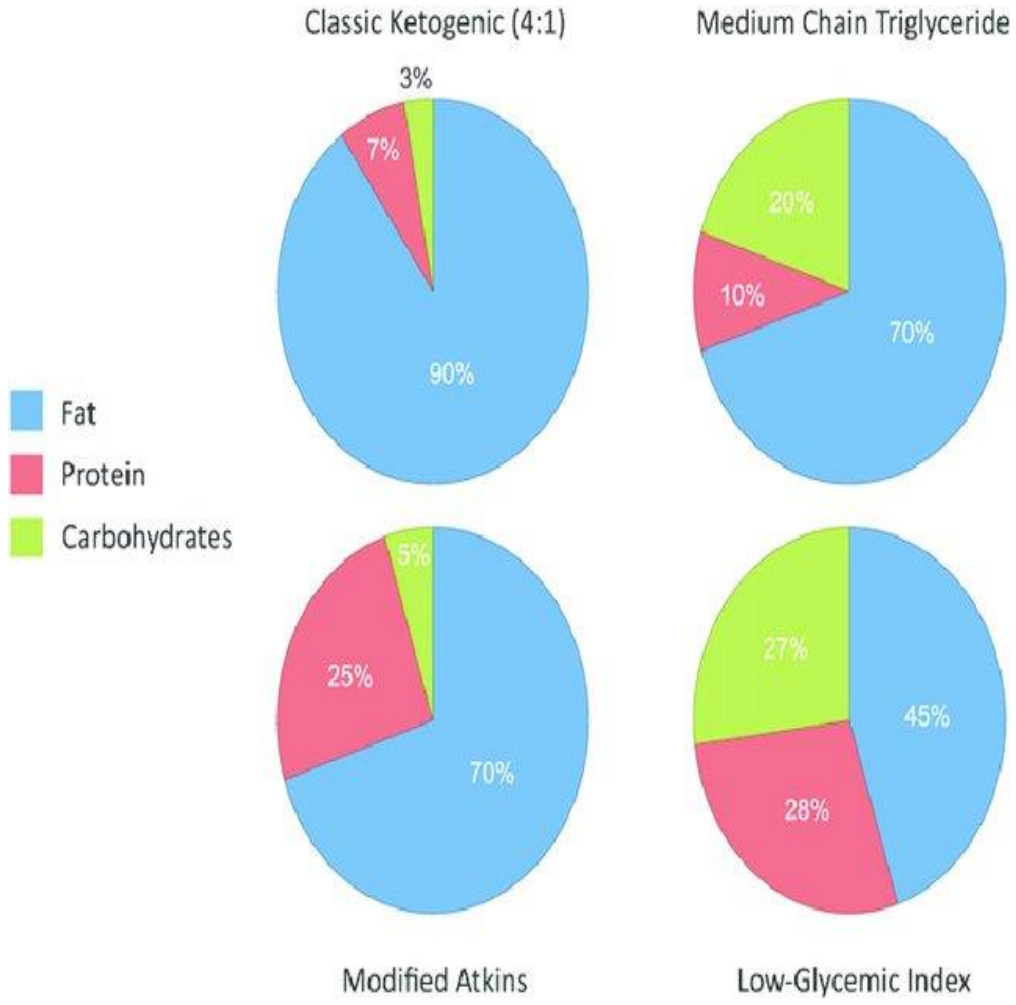


Figure 2.3 Comparison of fat, protein and carbohydrate intake in the four types of the ketogenic diet (Gano et al., 2014)

2.2.1 Ketogenic Diet Rich in Medium Chain Triglycerides.

The diet rich in MCTs are a variation of the KD in which MCTs are used as a fat component. This diet was described in 1971, indicating its similarity in the anticonvulsant effects with the classic KD (Gano et al., 2014). It was developed to achieve better palatability and higher consumption of carbohydrates and proteins since antiepileptic ketogenic levels were reached earlier. MCTs elicit a higher amount of ketone bodies per kilocalorie of energy than long-chain triglycerides (LCTs) (Reid et al., 2014) Medium chain fatty acids have between 6 and 12 carbon atoms.

Triglycerides derived from these fatty acids provide 25% fewer calories than LCT; triglycerides derived from medium chain fatty acids provide 6.8 cal/g and LCT, 9 cal/g (Sáyago-Ayerdi et al., 2008). These MCTs are more rapidly absorbed in the intestine than LCTs since more than 30% of MCTs are absorbed via the portal blood, unlike LCTs, which are absorbed via the lymphatics. In addition, they are metabolized faster, so instead of turning into fat, they are used to obtain energy from organs and muscles. The latter is because MCTs do not need carnitine as a transporter, generating an excess of acetyl-CoA that stimulates the production of ketone bodies (Pérez-Guisado, 2010).

This type of diet has been used as a treatment for epilepsy but also for weight loss because MCTs have been shown to have a more significant satiating effect due to the production of ketone bodies such as β-hydroxybutyrate, which have anorexigenic effects (Pérez-Guisado, 2010). In addition, these MCTs present greater thermogenesis, so the body burns more calories by consuming foods rich in them, thus benefiting weight loss (Sáyago-Ayerdi et al., 2008). That is why a diet rich in MCT would be beneficial both for getting into a state of ketosis in patients resistant to developing it and

allowing a greater intake of carbohydrates without leaving the ketogenic state (Pérez-Guisado, 2010). Some people have poor intestinal tolerability to these MCTs if they are ingested at high doses, so this could be a problem such as flatulence, diarrhea, stomach pain, and bloating..

2.2.2 Modified Atkins diet (MAD)

The MAD was designed to allow more liberalized intake of protein and calories. However, carbohydrate intake remains very limited, initially 10 g/day in children and 15 g/day in adults, although this amount can increase to 20-30 g/day after following the diet for a couple of months. Weight loss is not the main objective, although it is also often used for this purpose (Gano et al., 2014). It was mainly designed to demonstrate that refractory epilepsy could be controlled with this type of diet that is less restrictive than the classic KD (Vaccarezza et al., 2016).

Approximately 65% of the calories ingested come from fat sources, compared to 90% in the classic ketogenic diet. (Kossoff and Dorward, 2008). This makes the ketogenic ratio between fat and protein + carbohydrate 0.9:1, compared to 4:1 in the classic ketogenic diet. In addition, this is a diet with no caloric, fluid or protein restriction, leading to improved compliance with the diet (Vaccarezza et al., 2016).

As mentioned before, a modification of the classic Atkins diet was made to use this type of diet for the proposed purposes. This modification consisted of indefinitely limiting the amount of carbohydrates, unlike the classic Atkins diet, which is only limited in the induction phase. All types of carbohydrates are allowed, which can be given throughout the day or at one meal, with fiber being ignored in the total carbohydrate count (Kossoff and Dorward, 2008). first, a period is proposed in which 3 months it is assessed whether the diet is effective or if any type of complication arises. After this, the duration of the

treatment could be extended up to two years, with the possibility of increasing it for longer if the epileptic seizures reappear when the diet is interrupted (Vaccarezza et al., 2016).

2.2.3 Low glycemic index diet

The glycemic index (GI) measures the increase in circulating glucose in response to the ingestion of food. It evaluates how each gram of carbohydrate contained in a food raises glucose levels compared to the intake of pure glucose, which is assigned a GI of 100 (Gano et al., 2014).

This type of diet is less restrictive than the classic ketogenic diet as a higher intake of carbohydrate-containing foods is allowed. In addition, this type of diet in which foods with a low GI are consumed decreases insulin levels since foods rich in carbohydrates with a high GI stimulate its secretion. Another beneficial effect of this diet is that it allows greater appetite control. High GI foods can make the body unable to access stored metabolic fuels, leading to excessive hunger and overeating. For all these reasons, the low GI diet would be a good option for patients whose goal is to reduce body weight (Spieth et al., 2000).

This diet increases carbohydrate intake to 40-60 g/day but only allows the consumption of foods with a GI <50. In it, 60% of the calories ingested come from fat, and 20-30% of the calories would come from protein (Gano et al., 2014)

2.3 Insulin Resistance

Insulin resistance is a condition in which the body synthesis insulin hormone but does not use it properly(Wilcox, 2005). Insulin, a hormone made by the beta-cells of the pancreas, helps the body use glucose as energy through glycolysis(Fu et al., 2013). The body’s alimentary canal digests food into glucose and then travels in the bloodstream to cells throughout the body. Glucose in the blood is called blood glucose or blood sugar. As the blood

glucose level rises after a meal, the pancreas releases insulin to help cells take in and use the glucose(Fu et al., 2013). When people are insulin resistant, their muscle, fat, and liver cells do not respond appropriately to insulin. As a result, their bodies need more insulin to help glucose enter cells. The pancreas tries to keep up with this increased demand for insulin by producing more. Eventually, the pancreas fails to keep up with the body's need for insulin. Excess glucose builds up in the bloodstream, setting the stage for diabetes Thus, both blood sugar and insulin level can be high in people with insulin resistance (Wilcox, 2005).

Until recently, assessment of tissue insulin sensitivity and pancreatic beta-cell function was challenging. Measuring parameters such as pro-insulinemia, insulinemia or C-peptide was not common and involved technical problems. Over time, new specifications and laboratory tests have improved the perspective, and now reliable insulin sensitivity indicators are obtained from dynamic analyses of peripheral glucose utilization (Cohen et al., 2006). Some examples are the euglycemic insulin clamp, the homeostasis model assessment (HOMA: Homeostasis Model Assessment), the insulin-glucose ratio or the Bennett index. These tests are helpful in research but require cannulation or continuous intravenous infusion and, consequently, specialized personnel. They are challenging to apply in primary care where, due to infrastructure problems, it is always more practical to measure static parameters. In this sense, it has been shown that the combined determination of fasting insulinemia and triglyceridemia, despite not reporting peripheral glucose utilization, is a convenient formula to assess IR in epidemiological studies (Lorenzo et al., 2003). However, routine determination of insulinemia is not recommended in practice because to indirectly assess IR, much more accessible and cost-effective clinical parameters are probably sufficient.

2.4 Prediabetes.

Prediabetes is a serious condition in which blood sugar levels are higher than normal but have not yet reached high enough for T2DM to be diagnosed. Approximately 96 million adults in US(more than 1 in 3) have prediabetes. Among them, more than 80% do not know they have prediabetes. Prediabetes increases the Patient's risk of acquiring T2DM., heart disease and stroke (Boyle et al., 2010,Knowler et al., 2002, , Albright and Gregg, 2013). Prediabetes, a combination of excess body fat and insulin resistance, is an underlying a etiology of metabolic syndrome. It manifests as impaired fasting glucose and/or impaired glucose tolerance. (Mayans, 2015). Prediabetes is characterized by abnormal cellular responses to insulin. The pancreas makes more insulin, but it won't be able to keep up, and the blood sugar levels will rise, setting the stage for prediabetes and T2DM later in life (Albright and Gregg, 2013).

2.5 Overweight / Obesity

A classification that describes the weight of adults and the associated health risk in this context is shown in Table 2.2 modified according to the WHO from 2000.

Table 2.1: Classification of adult obesity according to BMI (Garrow and Webster, 1985, Wohlfahrt-Veje et al., 2014, Freedman et al., 2013)

category	**BMI [kg/m²]**	**risk of secondary diseases**
Underweight	<18.5	Low
Normal weight	18:5-24:9	Average
Overweight	25-29.9	Slightly increased
Grade I obesity	30-34.9	Elevated
Grade II obesity	35-39.9	High
Grade III obesity	≥40	Very High

The causes of overweight and obesity are multifactorial. They can be biological, psychosocial or environmental. The causes include family disposition (genetic causes), people's lifestyle, the constant availability of food, lack of sleep, stress, depressive illnesses, low social status, eating disorders, endocrine diseases, medication, but also immobilization, pregnancy and smoking cessation (Shai et al., 2008).

2.6 Dyslipidemias

A large number of components can influence the lipid metabolism change accordingly. In conjunction with the risk factors already explained (diabetes mellitus, hypertension, obesity, etc.), a disruption in lipid metabolism can lead to the sequelae of atherosclerosis, which in turn has a high mortality rate even in interaction with thrombotic changes(Kopin and Lowenstein, 2017). Several lipid disorders are known, with the features illustrated in Table 2.3, the lipid metabolism disorder can be indicated if plasma triglycerides and cholesterol are estimated.

Table 2.2: Classification of hyperlipidemias (Nelson, 2013)

Hypercholesterolemia	Plasma Cholesterol Concentration	> 200 Mg/Dl
Hypertriglyceridemia	Plasma Triglyceride Concentration	> 150 Mg/Dl
Combined Hyperlipidemia	Increase in Triglycerides and Cholesterol	

The causes of dyslipidemia can be divided into three groups and several mixed forms:

1. reactive-physiological forms.
2. secondary forms.
3. primary (hereditary) lipid metabolism disorders.

The reactive-physiological causes are related to a moderate, primarily temporary metabolic overload/disorder. They are characterized by an unhealthy diet or an unfavorably chosen lifestyle. Among other things, increased alcohol consumption and high-calorie food intake can result in hypertriglyceridemia. On the other hand, the development of hypercholesterolemia is favored by high fat intake in the form of saturated animal fats.

The secondary causes, on the other hand, are associated with an underlying disease that can lead either to hypertriglyceridemia (diabetes mellitus, metabolic syndrome, obesity, pregnancy, etc.) or to hypercholesterolemia (nephrotic syndrome, hypothyroidism, cholestasis, diabetes mellitus, etc.).

Finally, a group of lipid metabolism disorders is summarized under the primary forms for which molecular genetic analyzes are required. From an epidemiological point of view, more than 50% of the population aged >40 years living in western industrialized countries are affected by cholesterol levels >200 mg/dl (5.2 mmol/l), which again underlines the explosiveness of the current health situation (Brehm et al., 2003, Elkins et al., 2019).

2.7 Advantages of applying ketogenic diet.

The ketogenic diet has many advantages and is used to treat various pathologies including:-

2.7.1 Use of ketogenic diet as a treatment for epilepsy.

One of the most widespread uses of the ketogenic diet is for treating refractory or intractable epilepsy. Dr. Wilder in 1921, was the first to coin the term KD as he suggested that a high-fat, low-carbohydrate diet could maintain the state of ketosis necessary to prolong its antiepileptic properties (Sankaraneni and Singh, 2022).

2.7.2 The ketogenic diet and weight loss.

Obesity is one of the diseases whose prevalence has increased rapidly in recent years. It affects millions of people worldwide and is one of the main risk factors for cardiovascular diseases. Furthermore, dyslipidemia, hypertension, and diabetes contribute to metabolic syndrome (Paoli, 2014).The ketogenic diet was designed to treat epileptic seizures in children; however, as of 1970, it was used as a diet therapeutic proposal to treat weight loss. But it was much later when the efficacy of this diet for this purpose was demonstrated (Gutiérrez et al., 2013).

In other sense, one of the most critical aspects of the KD is the satiating effect it produces. As is known, carbohydrates by themselves and in large quantities could promote obesity. Therefore, a low-carbohydrate diet would help with weight loss; the fact that carbohydrates have a lesser satiating effect is of great relevance than proteins and lipids because the proteins and fats stay in the stomach longer than carbohydrates. In addition, when carbohydrates and fat are consumed simultaneously, fat accumulation is favoured, and when consumed with proteins, the obesity-inducing effect can be increased. Also, consuming fats and proteins stimulates cholecystokinin, a potent hormone suppressing appetite (Bekkouche et al., 2014). Acetone and β-hydroxybutyrate also contribute to the satiating effect by inhibiting the appetite centre (Deemer et al., 2020).

Another critical point is that when using a diet high in fat and protein, such as the ketogenic diet, the insulin levels in the blood are low. Therefore, the use of fat will be favoured, which will be beneficial; that is, it changes to a lipolytic metabolism that attacks fat deposition (Fabbrini and Klein, 2008).On the other hand, proteins have an anorexigenic effect superior to carbohydrates. This may be due to increased sensitivity of the central nervous system to leptin, an

appetite suppressant, and to decreased postprandial plasma concentrations of ghrelin, a hormone that stimulates appetite. Everything could be summed up in that ketosis produces a suppression of appetite that, together with the modifications that occur in the levels of hormones that influence appetite, could favour weight loss (Paoli et al., 2013a, Deemer et al., 2020).

In addition, fluctuations in plasma glucose concentrations are reduced in the KD due to the low GI of this diet. These fluctuations are more typical in diets rich in carbohydrates, In this manner, the KD is able to satiate and control appetite. (Fabbrini and Klein, 2008).It has been shown that consuming the same number of calories on a ketogenic and low-fat diet makes the KD more effective for weight loss because gluconeogenesis is a prominent process in ketogenic diets. Gluconeogenesis is an energetically expensive process since the endogenous breakdown of 110 g of protein, which is necessary to form 60-65 g of glucose, generates a loss of approximately 400-600 kcal/day (Gutiérrez et al., 2013) (figure 2.5).From a long-term perspective, the success of nutritional approaches depends on the weight regained over time. Recently, it has been shown that two short periods of ketogenic diet application separated by more extended periods of maintenance on the Mediterranean diet led to successful weight loss at the long-term and improvements in health risk factors without regaining weight lost while using this KD (Paoli, 2014).

2.7.3 The Ketogenic Diet and Diabetes Mellitus.

Diabetes mellitus is a severe universal health problem. There are two main types of diabetes (Al-Khalifa et al., 2009): Type I diabetes mellitus or insulin-dependent diabetes: which is caused by autoimmune destruction of pancreatic β cells, which leads to insulin deficiency (Ikegami et al., 2022).

four autoantibodies serve as markers of beta cell autoimmunity: islet cell antibodies (ICA, against cytoplasmic proteins in the beta cell), antibodies to glutamic acid decarboxylase (GAD-65), insulin autoantibodies (IAA), and IA2A, to protein tyrosine phosphatase. Autoantibodies against GAD 65 are found in 80% of patients with type 1 diabetes at clinical presentation. Presence of ICA and IA-2A at diagnosis for type 1 diabetes range from 69-90% and 5475%, respectively. IAA prevalence correlates inversely with age at onset of diabetes; it is usually the first marker in young children at risk for diabetes and found in approximately 70% of young children at time of diagnosis.

Type II diabetes mellitus: it is caused by an alteration in insulin secretion and or insulin resistance. This type of diabetes is the most common, occurring in approximately 90% of cases of diabetes mellitus. As mentioned, patients with type II diabetes mellitus have insulin resistance as their main characteristic, but this problem can also occur in the general population that does not have diabetes. One of the main characteristics of IR is the inability to absorb circulating glucose from muscle cells. Therefore, a person with insulin resistance diverts much of the carbohydrate in the diet to the liver, where it is converted to fat (de novo lipogenesis), instead of being oxidized for energy in skeletal muscle. This disorder of metabolism increases the risk of diabetes and cardiovascular disease. By restricting carbohydrates in the diet, Furthermore, in diabetes, ingested carbohydrates are absorbed primarily as glucose, causing an immediate rise in blood glucose level. When following a KD, triglycerides and proteins are mainly absorbed instead of glucose, thus this increase in blood glucose would be avoided (Al-Khalifa et al., 2009) (figure 2.5).

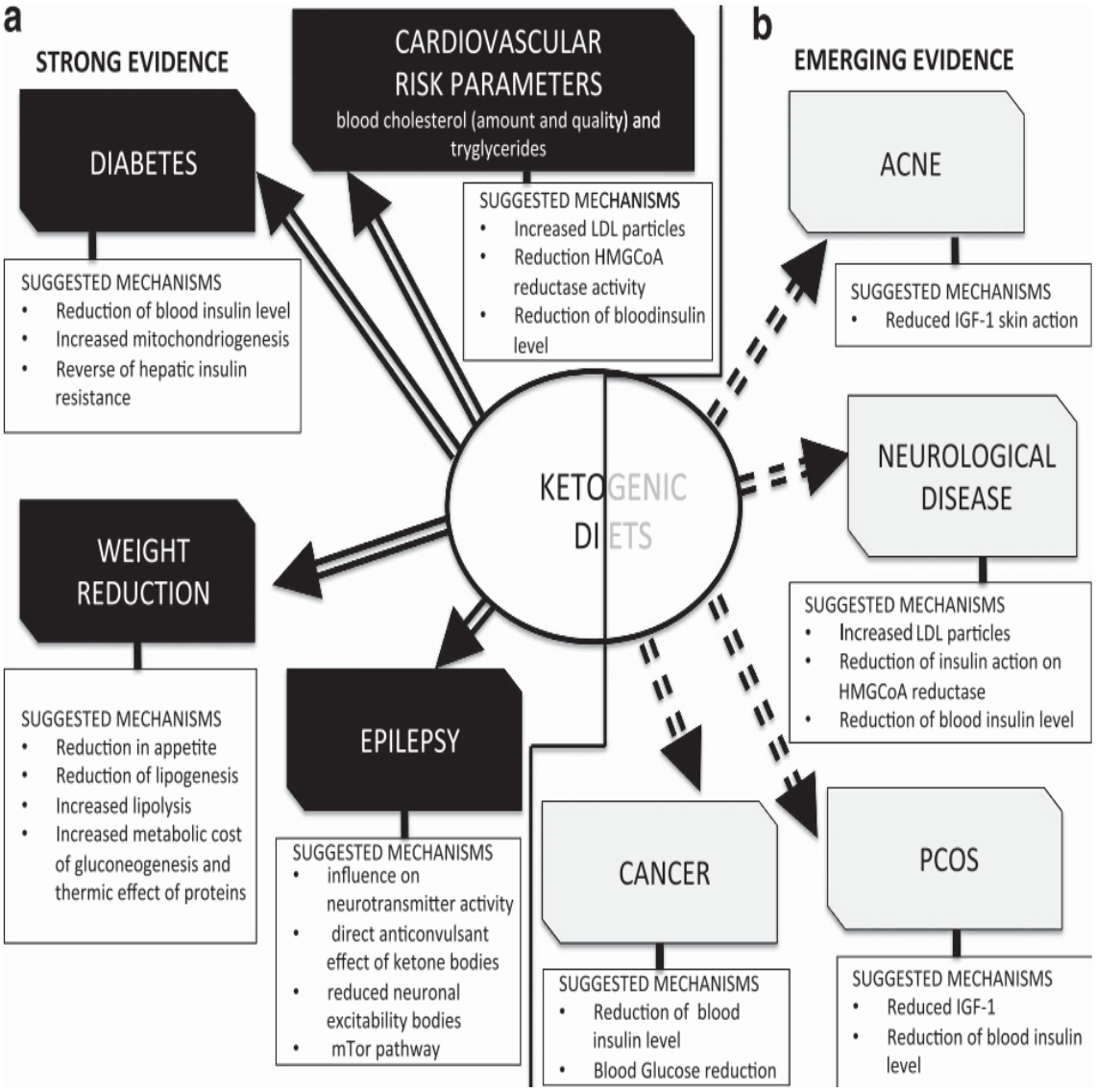


Figure 2.4 Suggested mechanisms of nutritional ketosis therapeutically in pathologies for which there exists strong (a) and emerging (b) evidence (Paoli et al., 2013b).

2.8 Disadvantages of using the ketogenic diet.

Contrary to all the advantages described above, the ketogenic diet is not suitable for everyone and may have some adverse effects.

2.8.1 General adverse effects.

In general, being a stricter diet than conventional diets, with the KD, there is a decreased contribution of vitamins and minerals, which does not cover the requirements of each person. This is due to a minimal consumption of fruits and vegetables, the main foods that provide these nutrients (Gutiérrez et al., 2013).

In addition, one of the most frequent adverse effects is constipation since fiber consumption is also minimal. Other gastrointestinal problems include vomiting, abdominal pain, diarrhoea, and taste disturbances(Flu, 2018). Cases of halitosis or bad breath and skin rash have also been reported (Bostock et al., 2020).

2.8.2 Ketogenic diet and ketogenic flu 'keto flu'.

Among the most apparent adverse effect of the ketogenic diet is the 'ketogenic flu' or 'keto flu', which consists of a set of transitory symptoms that usually occur during the first weeks since the body needs to adapt to ketones as its primary energy source. The symptoms that occur most frequently are: flu (44.5%), fatigue (17.82%), nausea (15.8%), dizziness (14.8%), decreased energy (9.9%), feeling faint (7.92%), heartbeat disturbances (5.9%), sore throat (5.9%), decreased appetite (4.9%), tremors (4.9%), and body aches (3.9%) (Bostock et al., 2020).

2.8.3 Ketogenic diet and kidney stones.

The presence of kidney stones has been associated with the use of the ketogenic diet and is an essential complication of it. These kidney stones occur

in 3-5% of patients and can be prevented with adequate hydration and avoiding medications that increase your risk. These medications are adrenocorticotropic hormone, acetazolamide, prednisone, and topiramate (Acharya et al., 2021). Kidney stones are usually composed of uric acid, calcium oxalate, or a mixture of calcium oxalate and phosphate/uric acid (Choi et al., 2010). Patients following the diet are at risk of hypercalciuria, acid urine, and low urinary citrate excretion.

Any evidence of crystalluria, hematuria, or increased seizures (this may be secondary to pain) while on a ketogenic diet should be followed by a renal ultrasound to look for stones. In the case of suffering from urolithiasis, treatment is individualized according to the type of stone. This allows patients who develop this pathology to continue with the ketogenic diet (Acharya et al., 2021)

2.8.4 The ketogenic diet and the lipid profile.

The ketogenic diet has been shown to have detrimental effects on the lipid profile of patients subjected to it. Increases in total cholesterol, triglycerides, and major lipoproteins containing atherogenic apolipoprotein B (low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) have been observed. In addition, the primary antiatherogenic apolipoprotein A (contained in high-density lipoprotein (HDL) showed a relevant reduction (Villalón Rodríguez, 2020).This is a significant problem as early atherosclerotic lesions, fatty streaks, and fibrous plaques could develop in adolescents' and young adults' aorta and coronary arteries (Kwiterovich Jr et al., 2003, O'Neill and Raggi, 2020) .

2.8.5 Ketogenic diet and cardiovascular disorders.

The use of the KD can alter cardiovascular functions due to the dyslipidemia that it can cause. An association of the ketogenic diet and left ventricular dilatation and systolic dysfunction has been observed, which appears to be related to selenium deficiency (Guzel et al., 2019).

The degree of ketosis may be related to increases in TNF-α, a risk factor for vascular inflammation, and protein carbonyls. Likewise, the increase in ketone bodies is associated with lipid peroxidation, another risk factor for cardiovascular diseases (Burkitt, 2020).

Elevated transaminase levels have been observed in some patients and may represent cardiac dysfunction and passive congestion of the liver. The biochemical changes produced in the ketogenic diet can alter the cardiac conduction of vulnerable patients, causing these patients to present a higher risk of ventricular arrhythmias and sudden death since an increase in the QT interval would occur. This interval measures ventricular depolarization and repolarization, that is, ventricular systole, reflecting the duration of ventricular contraction (Sudhakaran et al., 2020).

In the glycolytic metabolism of glucose, methylglyoxal (CH3COCHO) is highly reactive in glycation-type reactions. It gives rise to various glycation end products (AGEs), which can cause structural modifications in some molecules. The ketones produced in the KD can modify proteins through mechanisms very similar to those related to glucose 'toxicity'. Because following a ketogenic diet increases blood ketone levels and exceeds glucose levels, some authors suggest that the ketogenic diet has long-term problems and that this type of diet should not be recommended (Burkitt, 2020). To prevent this problem, patients should be evaluated before and during therapy with electrocardiograms and echocardiograms (Best et al., 2000, Sudhakaran et al., 2020).

2.7.6 The ketogenic diet and liver problems.

The safety of the ketogenic diet has been raised; primarily when used long-term, due to possible liver damage it can cause. Various studies have confirmed the occurrence of steatosis and liver inflammation in mice (Watanabe et al., 2020). Also, increased liver enzyme levels have been observed in isolated cases of studies in children. However, the liver injury may be due to the use of valproate and not the ketogenic diet itself. Furthermore, there is significant evidence that the ketogenic diet could play a hepatoprotective role when applied to obese patients with metabolic syndrome (Watanabe et al., 2020).

The ketogenic diet can induce hepatotoxicity due to a deficiency of carnitine. In the ketogenic diet, this carnitine deficiency can occur since it is included especially in red meat and milk; the intake of red meat is very restricted and that of milk is totally restricted. In addition, the use of carnitine can be increased in the ketogenic diet since it is high in long-chain fatty acids. For all this, there may be an additive effect causing a significant carnitine deficiency that can cause or aggravate hepatotoxicity (Roehl et al., 2019).

2.7.7 Prurigo pigmentosa.

Several prurigo pigmentosa have also been reported in patients due to the KD. Prurigo pigmentosa is a rare skin disorder that causes inflammation. This pathology progresses through two distinct phases, both clinically and histologically; First, there is an acute inflammatory phase followed by a hyperpigmented phase that is usually more chronic. Patients develop pruritic papules and papulovesicular on the neck, chest and back. These progress to crusted papules and ultimately to hyperpigmented macules. The mechanism of pathogenicity is not well understood, but skin inflammation may be due to exogenous stressors such as ketosis. To confirm this, high levels of pro-inflammatory cytokines such as interleukin-6 (IL-6) and TNF were shown in

ketotic cows, showing a positive correlation with high blood concentrations of non-esterified fatty acids (Lonowski and Levins, 2020). If pruritus pigmentosa appears when using the KD, its use should be stopped, and antibiotics that affect neutrophil chemotaxes, such as minocycline and doxycycline, should be administered (Lonowski and Levins, 2020).

2.7.8 Contraindications of the ketogenic diet in various pathologies.

The ketogenic diet can worsen some metabolic diseases such as porphyria, organic aciduria, pyruvate carboxylase deficiency, fatty acid oxidation defects, primary ketogenesis/ketolysis defects, etc. This is because using the KD could worsen the baseline situation of these pathologies (Ramirez-Camacho et al., 2011). As fatty acid metabolism disorders and organic aciduria occur in these diseases, the ketogenic diet would not be a good option for this patient since fat is the primary energy source (Oliveira et al., 2018).

METHODOLOGY AND RESEARCH DESIGN

3. METHODOLOGY AND RESEARCH DESIGN

3.1. MATERIALS

3.1.1 Equipments

The instruments and their manufacturers used in the present study were summarized in Table (3.1).

Table 3.1: Instruments and their manufacturers used in the present study.

No.	Instruments	Company	Country
1	Analytical balance	Mettler PM200	USA
2	Centrifuge	Centromix	Spain
3	COBAS E411	Roche	Germany
4	COBAS Ingtra 400	Roche	Japan
5	Deep freezer	Sanyo	Japan
6	ELISA reader	BioTek	USA
7	ELISA washer	BioTek	USA
8	Micropipettes	Bibby Sterilin	UK
9	Vortex	Genie	USA
10	Water bath	Memmert	Germany
11	Spectrophotometer	Shimadzu	Japan

3.1.2 Kits

The following kits were used in the present study

Table 3.2: Kits used in the present study.

No.	Kits	Company	Catalogue No
1	Interleukin-10 ELISA kit	eBioscience	15561047
2	Elecsys C-Peptide	Roche	03184919190
3	ALP IFCC Gen.2	Roche	03333752
4	Alanine Aminotransferase	Roche	20764957
5	Aspartate Aminotransferase	Roche	20764949
6	Cholesterol Gen.2	Roche	03039773
7	Creatinine Jaffé	Roche	20764345
8	Glucose HK	Roche	20767131
9	HDL-Cholesterol plus	Roche	03038637 322
10	LDL-Cholesterol plus	Roche	03038866
11	Uric Acid	Roche	20756296
12	Urea/BUN	Roche	20763039 322

3.2 METHODS

3.2.1 Study design

The study was conducted in two phases, pre-phase and post-phase. The pre-phase sample was analyzed before the start of KD, and the post-phase sample was analyzed after 13 weeks of KD. In both phases, the samples were analyzed for BMI, Glucose, C Peptide, lipid profile, renal function tests (Urea,

Uric acid, BUN and Creatinine) and Liver function tests (ALT, AST, and ALP). All participants were provided with a questionnaire or observation sheet for daily monitoring.

3.2.2 Participants

A total of 58 individuals, including 48 males and 10 females, were recruited for this study. The samples were drawn in pre-phase and post-phase in public. After blood collection, the samples were used to investigate glucose, C Peptide, HOMA-IR, HOMA S%, and HOMA B%, total cholesterol, HDL, LDL, VLDL, triglycerides, urea, uric acid, ALT, AST, and creatinine.

3.2.3 Anthropometric measurements

Body weight was measured to the nearest 0.1 kg using a Mettler Toledo balance having model number BBA231-3BC300A/C. Body mass index (BMI) was calculated in kg/m2 (Frankel and Staeheli, 1992)

3.2.4 Laboratory Analysis

The blood samples were collected and centrifuged at 2000 RPM for 10 min to obtain serum. By Roche Integra 400 analyzer, we did the measurements of creatinine, urea, uric acid, BUN, ALP, AST, ALT , lipid profile, FBS, and C-peptide tests. MDA and NO determination done manually and IL10 were measured by an ELISA kit bought from ElabScience, USA.

Moreover, HOMA2 Calculator version 2.2.3 is used to calculate HOMA IR, HOMA S%, and HOMA B% (Research, 2020, Holman et al., 2004). the participants are grouped into the prediabetes and control groups based on the HOMA IR results. If the HOMA is higher than 2.0, it's considered prediabetes (Salgado et al., 2010).

3.2.4.1 Estimation of Biochemical Parameters by Roche Integra 400 analyser.

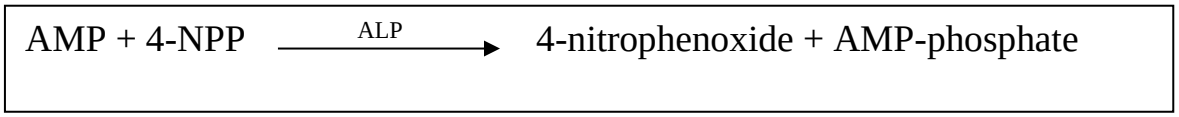
After measuring calibrator and quality control, serum samples were placed in the sample area, then the cobas analyser was run the tests. Results obtained after 5 minutes from starting time. The analyzer automatically calculated the analyte concentration of each sample in mg/dl.

3.2.4.1.1 Estimation of Alkaline Phosphatase

ALP refers to a group of phosphatases (pH optimum approximately 10) found in almost every tissue in the body. Most alkaline phosphatase in normal adult serum is from the liver or biliary tract. Normal alkaline phosphatase levels are age dependent with young children and adolescents having much higher levels than adults. Adult males tend to have higher levels than females, but pregnant females have increased levels due to placental secretion of alkaline phosphatase. Elevation of alkaline phosphatase levels occurs in diseases such as hepatitis, cirrhosis, malignancy, chemical toxicity, and in bone diseases such as metastatic carcinoma, rickets, Paget's disease, and osteomalacia. Moderate increases in serum alkaline phosphatase levels have been observed in Hodgkin's disease, congestive heart failure, ulcerative colitis, regional enteritis, and intra-abdominal bacterial infections. Normal alkaline phosphatase levels are elevated during periods of active bone growth, for example, in young children and adolescents (Sharma et al., 2014).

Test principle

According to the recommended reference method of the International Federation of Clinical Chemistry (IFCC). ALP hydrolyzes the colorless 4-nitrophenylphosphate ester (4-NPP) to 4-nitrophenoxide and phosphate. The product of enzyme hydrolysis, 4-nitrophenoxide, has a yellow color at the pH of the reaction. 2-Amino-2-methyl-1-propanol (AMP) functions as the phosphate acceptor and buffer (Tietz, 1983).



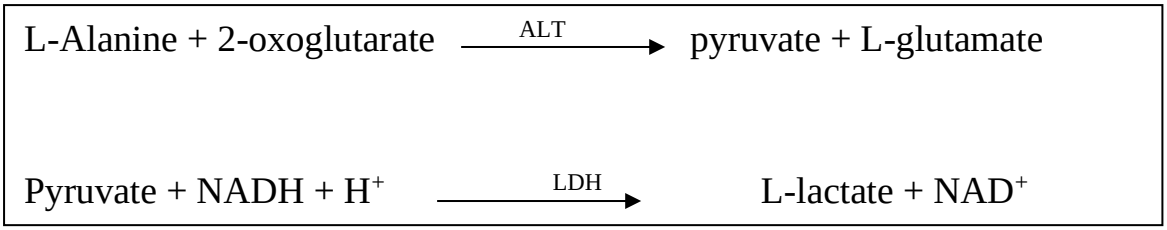
The rate of 4-nitrophenoxide formation is directly proportional to the catalytic ALP activity. It is determined by measuring the increase in absorbance at 409 nm.

3.2.4.1.2 Estimation of ALT

The enzyme ALT has been widely reported as present in a variety of tissues. The major source of ALT is the liver, which has led to the measurement of ALT activity for the diagnosis of hepatic diseases. Elevated serum ALT is found in hepatitis, cirrhosis, obstructive jaundice, carcinoma of the liver, and chronic alcohol abuse. and chronic alcohol abuse. ALT is only slightly elevated in patients who have an uncomplicated myocardial infarction. Although both serum AST and ALT become elevated whenever disease processes affect liver cell integrity, ALT is the more liver-specific enzyme. Moreover, elevations of ALT activity persist longer than elevations of AST activity. In patients with vitamin B6 deficiency, serum aminotransferase activity may be decreased. The apparent reduction in aminotransferase activity may be related to decreased pyridoxal phosphate, the prosthetic group for aminotransferases, resulting in an increase in the ratio of apoenzyme to holoenzyme(Pradeep et al., 2019).

Test principle

ALT catalyzes the reaction between L-alanine and 2-oxoglutarate. The pyruvate formed is reduced by NADH in a reaction catalyzed by lactate dehydrogenase (LDH) to form L-lactate and NAD+ (Bergmeyer et al., 1986a).



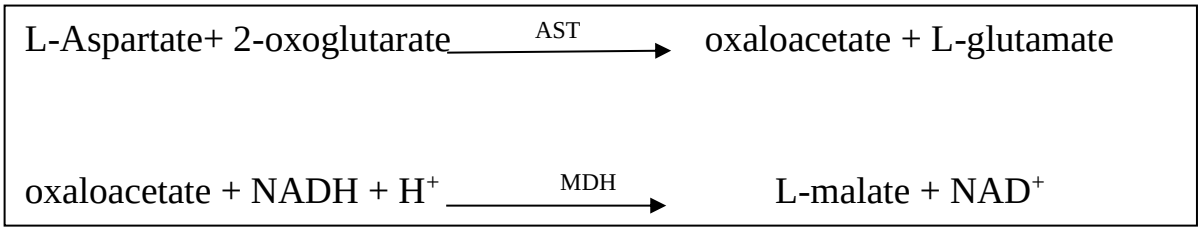
The rate of the NADH oxidation is directly proportional to the catalytic ALT activity. It is determined by measuring the decrease in absorbance at 340 nm

3.2.4.1.3 Estimation of AST

The enzyme AST is widely distributed in tissue, principally hepatic, cardiac, muscle, and kidney. Elevated serum levels are found in diseases involving these tissues. Hepatobiliary diseases, such as cirrhosis, metastatic carcinoma, and viral hepatitis also increase serum AST levels. Following myocardial infarction, serum AST is elevated and reaches a peak two days after onset. In patients undergoing renal dialysis or those with vitamin B6 deficiency, serum AST may be decreased. The apparent reduction in AST may be related to decreased pyridoxal phosphate, the prosthetic group for AST, resulting in an increase in the ratio of apoenzyme to holoenzyme. Two isoenzymes of AST have been detected, cytoplasmic and mitochondrial. Only the cytoplasmic isoenzyme occurs in normal serum, while the mitochondrial, together with the cytoplasmic isoenzyme, has been detected in the serum of patients with coronary and hepatobiliary disease (Murray and Kaplan, 1984, Moss et al., 1987).

Test principle

AST in the sample catalyzes the transfer of an amino group between L-aspartate and 2-oxoglutarate to form oxaloacetate and L-glutamate. The oxaloacetate then reacts with NADH, in the presence of malate dehydrogenase (MDH), to form NAD^+ (Bergmeyer et al., 1986b, Tietz, 1995).



The rate of the NADH oxidation is directly proportional to the catalytic AST activity. It is determined by measuring the decrease in absorbance at 340 nm.

3.2.4.1.4 Estimation of Cholesterol

Measurements of serum cholesterol levels are important in the diagnosis and classification of hyperlipoproteinemias. Elevated cholesterol levels may occur with hypothyroidism, nephrotic syndrome, diabetes, and various liver diseases. There is a correlation between elevated serum cholesterol levels and the incidence of coronary artery diseases. Normal cholesterol levels are affected by stress, diet, age, gender, hormonal balance, and pregnancy (Schade et al., 2020).

Test principle

Enzymatic, colorimetric method with cholesterol esterase, cholesterol oxidase, and 4-aminoantipyrine. Cholesterol esterase (CE) hydrolyzes cholesterol esters to form free cholesterol and fatty acids. Cholesterol oxidase (CHOD) then catalyzes the oxidation of cholesterol to form cholest-4-ene-3-one and H_2O_2. In presence of peroxidase (POD), the hydrogen peroxide formed effects the oxidative coupling of phenol and 4-amino-antipyrine (4-AAP) to form a red-colored quinoneimine dye (Allain et al., 1974).

Cholesterol esters + H_2O $\xrightarrow{CE}$ cholesterol + fatty acids
Cholesterol + O_2 $\xrightarrow{CHOD}$ cholest-4-ene-3-one + H_2O_2
2 H_2O_2 + 4-AAP + phenol $\xrightarrow{POD}$ quinoneimine dye + 4 H_2O

The color intensity of the red quinoneimine dye formed is directly proportional to the cholesterol concentration. It is determined by measuring the increase in absorbance at 520 nm.

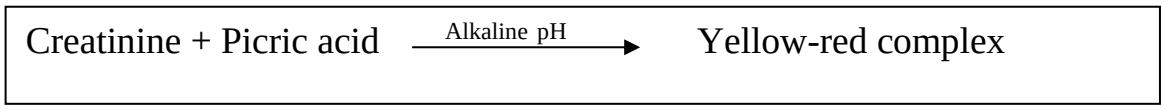
3.2.4.1.5 Estimation of Creatinine

Serum creatinine is a waste product formed by the spontaneous dehydration of body creatine. Most of the body creatine is found in muscle tissue where it is present as creatine phosphate and serves as a high energy storage reservoir for conversion to adenosine triphosphate. The rate of creatinine formation is fairly constant with 1 to 2 percent of the body creatine being converted to creatinine every 24 hours. Serum creatinine and urea levels are elevated in patients with renal malfunction, especially decreased glomerular filtration. In the early stages of kidney damage, the rise in the serum urea levels usually precedes the increase in serum creatinine. The advantage is offset by

the fact that serum urea levels are affected by factors such as diet, degree of hydration and protein metabolism. (Delanaye et al., 2017).

Test principle

Buffered kinetic Jaffé reaction without deproteinization. Compensated for serum/plasma. In alkaline solution creatinine reacts with picrate to form a yellow-red adduct (Jaffé, 1886, Fabiny and Ertingshausen, 1971, Cook, 1975).



The rate of the dye formation (color intensity) is directly proportional to the creatinine concentration in the specimen. It is determined by measuring the increase in absorbance at 512 nm. Serum and plasma samples contain proteins which react non-specifically in the Jaffé method. For compensation of serum and plasma results, values are automatically corrected by -18 µmol/L (-0.2 mg/dL).

3.2.4.1.6 Estimation of Glucose

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. (Giugliano et al., 2008, Von Ah Morano et al., 2020)

Test principle

Enzymatic reference method with hexokinase. Hexokinase (HK) catalyzes the phosphorylation of glucose by ATP to form glucose-6-phosphate and ADP. To follow the reaction, a second enzyme, glucose-6-phosphate dehydrogenase (G6PDH) is used to catalyze oxidation of glucose-6-phosphate by $NADP^+$ to form NADPH (Neeley, 1972, Bondar and Mead, 1974)

D-glucose + ATP $\xrightarrow{HK}$ D-glucose-6-phosphate + ADP

D-glucose-6-phosphate + $NADP^+$ $\xrightarrow{G6PDH}$ D-6-phosphogluconate + NADPH + H^+

The concentration of the NADPH formed is directly proportional to the glucose concentration. It is determined by measuring the increase in absorbance at 340 nm.

3.2.4.1.7 Estimation of HDL-Cholesterol

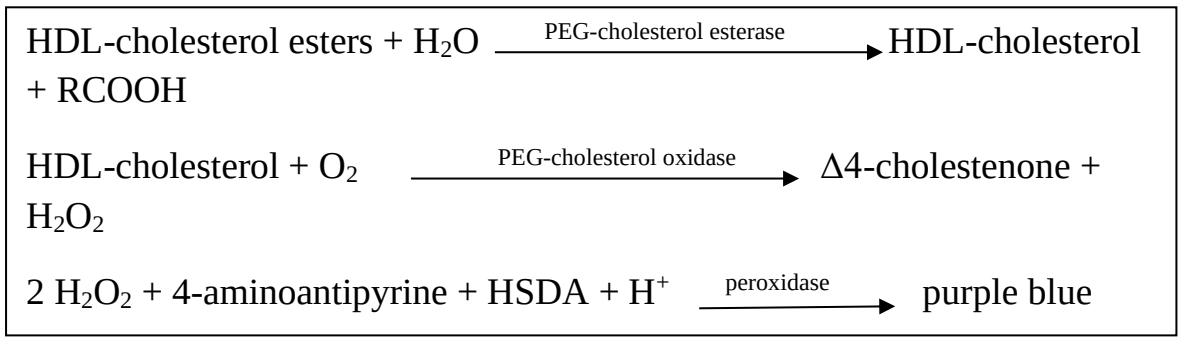
HDL are responsible for the reverse transport of cholesterol from the peripheral cells to the liver. Here, cholesterol is transformed to bile acids which are excreted into the intestine via the biliary tract. Monitoring of HDL-cholesterol in serum is of clinical importance since an inverse correlation exists between serum HDL-cholesterol concentrations and the risk of atherosclerotic disease. Elevated HDL-cholesterol concentrations are protective against coronary heart disease, while reduced HDL-cholesterol concentrations, particularly in conjunction with elevated triglycerides, increase the cardiovascular risk (Rader and Hovingh, 2014).

A variety of methods are available to determine HDL-cholesterol, including ultracentrifugation, electrophoresis, HPLC, and precipitation based methods. Of these, precipitation-based methods are used routinely. HDL-cholesterol is first separated by precipitating apoprotein B-containing lipoproteins from serum by using a combination of a polyanion and a divalent cation, such as dextran sulfate/magnesium chloride or phosphotungstate/magnesium chloride. Such precipitation-based methods are time-consuming and not amenable to automated analysis(Hafiane and Genest, 2015). Thus, there is a great clinical need for a convenient and reliable method for measuring HDL-cholesterol in serum without any pretreatment. Several approaches for direct measurement of HDL-cholesterol in serum have been proposed, including the use of magnetically responsive particles as polyanion-metal combinations and the use of polyethylene glycol (PEG) with anti-apoprotein B and anti-apoprotein CIII antibodies (Miller et al., 2010). The COBAS INTEGRA HDL-Cholesterol plus 2nd generation cassette is designed to the direct specific determination of HDL-cholesterol in the presence of LDL, VLDL, and chylomicrons. No sample pretreatment step is required (Tiyyagura and Smith, 2006).

Test principle

Homogeneous enzymatic colorimetric assay. In the presence of magnesium sulfate and dextran sulfate, water-soluble complexes with LDL, VLDL, and chylomicrons are formed which are resistant to PEG-modified enzymes. The cholesterol concentration of HDL-cholesterol is determined enzymatically by cholesterol esterase and cholesterol oxidase coupled with PEG to the amino groups (approximately 40%). Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by cholesterol esterase. In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to Δ4-

cholestenone and hydrogen peroxide. This direct assay meets the 1995 NCEP goals of 13% total analytical error (Warnick et al., 2001).



The color intensity of the blue quinoneimine dye formed is directly proportional to the HDL-cholesterol concentration. It is determined by measuring the increase in absorbance at 583 nm.

3.2.4.1.8 Estimation of LDL-Cholesterol

LDL play a key role in causing and influencing the progression of atherosclerosis and coronary sclerosis in particular. The LDLs are derived from VLDLs rich in triglycerides by the action of various lipolytic enzymes and are synthesized in the liver. The elimination of LDL from plasma takes place mainly by liver parenchymal cells via specific LDL receptors. Elevated LDL concentrations in blood and an increase in their residence time coupled with an increase in the biological modification rate results in the destruction of the endothelial function and a higher LDL-cholesterol uptake in the monocyte/macrophage system as well as by smooth muscle cells in vessel walls. The majority of cholesterol stored in atherosclerotic plaques originates from LDL (Packard et al., 2020). The LDL-cholesterol value is the most

powerful clinical predictor among all of the single parameters with respect to coronary atherosclerosis. Therefore, therapies focusing on lipid reduction primarily target the reduction of LDL-cholesterol which is then expressed in an improvement of the endothelial function, prevention of atherosclerosis and reducing its progression as well as preventing plaque rupture (Tada et al., 2018).Various methods are available for the determination of LDL-cholesterol such as ultracentrifugation as the reference method, lipoprotein electrophoresis and precipitation methods. In the precipitation methods apolipoprotein B-containing LDL-cholesterol is, for example, precipitated using either polyvinyl sulfate, dextran sulfate or polycyclic anions. The LDL-cholesterol content is usually calculated from the difference between total cholesterol and cholesterol in the remainder (VLDLand HDL-cholesterol) in the supernatant after precipitation with polyvinyl sulfate and dextran sulfate. Lipid Research Clinics recommend a combination of ultracentrifugation and precipitation methods using polyanions in the presence of divalent cations. The precipitation methods are time-consuming, cannot be automated and are susceptible to interference by hyperlipidemic serum, particularly at high concentrations of free fatty acids. A more recent method is based on the determination of LDL-cholesterol after the sample is subjected to immunoadsorption and centrifugation (Chary and Hedayati, 2022).The calculation of the LDL-cholesterol concentration according to Friedewald's formula is commonly practiced. The formula is based on two cholesterol determinations, one triglyceride determination as well as precipitation of the HDL particles and presumes that a direct relationship exists between VLDL-cholesterol and triglycerides in fasting blood samples. Even in the presence of small amounts of chylomicrons or abnormal lipoproteins, the formula gives rise to falsely low LDL-cholesterol values (Friedewald et al., 1972).

For this reason a great need exists for a simple and reliable method for the determination of LDL-cholesterol without any preparatory steps or calculation.

Test principle

Homogeneous enzymatic colorimetric assay. This automated method for the direct determination of LDL-cholesterol takes advantage of the selective micellary solubilization of LDL-cholesterol by a nonionic detergent and the interaction of a sugar compound and lipoproteins (VLDL and chylomicrons). When a detergent is included in the enzymatic method for cholesterol determination (cholesterol esterase cholesterol oxidase coupling reaction), the relative reactivities of cholesterol in the lipoprotein fractions increase in this order: HDL < chylomicrons < VLDL < LDL. In the presence of Mg^{++}, a sugar compound markedly reduces the enzymatic reaction of the cholesterol measurement in VLDL and chylomicrons. The combination of a sugar compound with detergent enables the selective determination of LDL-cholesterol in serum. In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to Δ4-cholestenone and hydrogen peroxide. This direct assay meets the 1995 National Cholesterol Education Program (NCEP) goals of <4% Total CV, bias ≤4% versus reference method, and ≤12 % total analytical error (Sugiuchi et al., 1998).

The color intensity of the blue quinoneimine dye formed is directly proportional to the LDL-cholesterol concentration. It is determined by measuring the increase in absorbance at 583 nm.

LDL-cholesterol ester + H_2O $\xrightarrow{\text{detergent cholesterol esterase}}$ cholesterol + free fatty acid (selective micellary solubilization)

LDL-cholesterol + O_2 $\xrightarrow{\text{cholesterol oxidase}}$ Δ4-cholestenone + H_2O_2

2 H_2O_2 + 4-aminoantipyrine + HSDA + H^+ + H_2O $\xrightarrow{\text{peroxidase}}$ purple blue pigment + 5 H_2O

3.2.4.1.9 Estimation of Triglycerides/ VLDL

Triglycerides are the main lipids present in human plasma; the others are cholesterol, phospholipids, and non-esterified fatty acids. They are formed in the intestinal mucosa by the esterification of glycerol and free fatty acids. They are then released into the mesenteric lymphatics and distributed to most tissues for storage. Triglycerides are the main storage lipids in humans, where they constitute about 95% of adipose tissue lipids (Stone, 2022). Elevated levels of triglycerides have been associated with high risk in severe atherosclerosis. High triglyceride levels and hyperlipidemia in general can be an inherited trait or can be secondary to disorders including diabetes mellitus, nephrosis, biliary obstruction, and metabolic disorders associated with endocrine disturbances (Lechner et al., 2020).

Test principle Enzymatic, colorimetric method (GPO/PAP) with glycerol phosphate oxidase and 4-aminophenazone. Triglycerides are hydrolyzed by lipoprotein lipase (LPL) to glycerol and fatty acids. Glycerol is then phosphorylated to glycerol-3-phosphate by ATP in a reaction catalyzed by

glycerol kinase (GK). The oxidation of glycerol-3-phosphate is catalyzed by glycerol phosphate oxidase (GPO) to form dihydroxyacetone phosphate and hydrogen peroxide (H_2O_2) (Fossati and Prencipe, 1982, McGowan et al., 1983).

Triglycerides $\xrightarrow{LPL}$ glycerol + fatty acids

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

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

2 H_2O_2 + 4-aminophenazone + 4-chlorophenol $\xrightarrow{POD}$ quinoneimine dye +4H_2O

In the presence of peroxidase (POD), hydrogen peroxide effects the oxidative coupling of 4-chlorophenol and 4-aminophenazone to form a red-colored quinoneimine dye, which is measured at 512 nm. The increase in absorbance is directly proportional to the concentration of triglycerides in the sample.

VLDL can be determined from the measured triglycerides via: VLDL = TG/5.

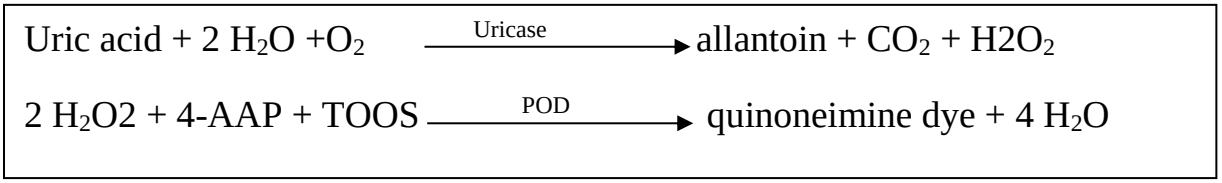
3.2.4.1.10 Estimation of Uric Acid

Uric acid is the major end product of purine metabolism and is one of the components of the nonprotein nitrogen fraction in plasma. Most uric acid formation occurs in the liver and is derived either from ingested or endogenous nucleoproteins (Furuhashi, 2020). Approximately half of the total uric acid in the body is eliminated daily by urinary excretion and destruction in the intestinal tract. Numerous disease states and physiological conditions are associated with alterations in serum uric acid concentrations. Increased levels are more frequent. Serum uric acid levels are characteristically elevated in gout, a disorder involving either uric acid synthesis or excretion. Other common

etiologies of hyperuricemia include renal dysfunction, ketoacidosis, glucose-6-phosphate deficiency, and Lesch-Nyhan syndrome. Decreased uric acid levels have been described in renal tubular absorption defects, Hodgkin's disease, bronchogenic carcinoma, severe hepatocellular disease, and xanthinuria (Battelli et al., 2018, Dalbeth et al., 2019).

Test principle

Enzymatic colorimetric test with uricase and 4-aminoantipyrine. In the initial step uric acid is oxidized in a reaction catalyzed by uricase. The hydrogen peroxide formed reacts with N-ethyl-N-(2-hydroxy-3-sulfopropyl)-m-toluidine (TOOS) and 4-amino-antipyrine (4-AAP) in the presence of peroxidase (POD) and forms a red quinoneimine dye (Barham and Trinder, 1972, Tamaoku et al., 1982).



The color intensity of the quinoneimine formed is directly proportional to the uric acid concentration and is determined by measuring the increase in absorbance at 520 nm. The addition of ascorbate oxidase prevents interference by ascorbic acid.

3.2.4.1.11 Estimation of Urea/BUN

Urea is the major end product of protein nitrogen metabolism. It is synthesized by the urea cycle in the liver from ammonia which is produced by amino acid deamination. Urea is excreted mostly by the kidneys but minimal amounts are also excreted in sweat and degraded in the intestines by bacterial action (Getahun et al., 2019). Determination of blood urea nitrogen is the most widely used screening test for renal function (Pundir et al., 2019). When used in conjunction with serum creatinine determinations it can aid in the differential diagnosis of the three types of azotemia: prerenal, renal, and postrenal. Elevations in blood urea nitrogen concentration are seen in inadequate renal perfusion, shock, diminished blood volume (prerenal causes), chronic nephritis, nephrosclerosis, tubular necrosis, glomerular-nephritis (renal causes), and urinary tract obstruction (postrenal causes). Transient elevations may also be seen during periods of high protein intake. Unpredictable levels occur with liver diseases (Han et al., 2020).

Test principle

Kinetic test with urease and glutamate dehydrogenase. Urea is hydrolyzed by urease to form ammonium and carbonate. In the second reaction 2-oxoglutarate reacts with ammonium in the presence of glutamate dehydrogenase (GLDH) and the coenzyme NADH to produce L-glutamate. In this reaction two moles of NADH are oxidized to NAD for each mole of urea hydrolyzed.(Tiffany et al., 1972, Sampson et al., 1980)

$$\text{Urea} + 2\,H_2O \xrightarrow{\text{Urease}} 2\,NH_4^{+} + CO_3^{2-}$$

$$NH4^{+} + \text{2-oxoglutarate} + NADH \xrightarrow{\text{GLDH}} \text{L-glutamate} + NAD^{+} + H_2O$$

The rate of decrease in the NADH concentration is directly proportional to the urea concentration in the specimen. It is determined by measuring the absorbance at 340 nm.

3.2.4.2 Estimation of C peptide Parameters by Roche Cobas E411

After measuring calibrator and quality control, serum samples were placed in the sample area, then the cobas analyser was run the tests. Results obtained after 5 minutes from starting time. The analyzer automatically calculated the analyte concentration of each sample in ng/L.

Summary

C-peptide is a single chain 31-amino acid (AA 33-63) polypeptide connecting the insulin A chain with the B chain in the proinsulin molecule. It has a molecular weight of approximately 3021 Da (Venugopal et al., 2022). The proteolytic cleavage of the precursor proinsulin results in the two molecules insulin and C-peptide. Both are secreted in equimolar amounts and released into circulation via the portal vein. As half of the insulin, but almost none of the C-peptide is extracted in the liver, C-peptide has a longer half-life (about 35 minutes) than insulin. 5 to 10 times higher concentration of C-peptide persist in the peripheral circulation, and these levels fluctuate less than insulin (Venugopal et al., 2022). C-peptide is removed from the circulation by the kidneys and degraded, with a fraction excreted unchanged in the urine. The concentration in urine is about 10-20 fold higher than in serum (Jones and

Hattersley, 2013). In the past, C-Peptide has been considered biologically inactive. However, recent studies have demonstrated that it is capable of eliciting molecular and physiological effects suggesting that C-peptide is in fact a bioactive peptide (Yosten and Kolar, 2015). There is evidence that C-peptide replacement, together with insulin administration, may prevent the development or retard the progression of long-term complications in type 1 diabetes (Forst et al., 2002, Maddaloni et al., 2022).

Test principle

Sandwich principle.

Total duration of assay: 18 minutes.

1st incubation: 20 µL of sample, a biotinylated monoclonal C-peptide-specific antibody, and a monoclonal C-peptide-specific antibody labeled with a ruthenium complex a react to form a sandwich complex.

2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.

The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

Results are determined via a calibration curve which is instrument specifically generated by 2-point calibration and a master curve provided via the reagent barcode or e-barcode.

3.2.4.3 Estimation of IL-10

Assay Principle of IL-10 (ELISA)

An anti-human IL-10 coating Ab is adsorbed onto the microwells. Human IL-10 present in the sample or standard binds to Abs adsorbed to the microwells. A biotin-conjugated anti-human IL-10 Ab is added and binds to human IL-10 captured by the first Ab. Following incubation unbound biotin-conjugated anti-human IL-10 Ab is removed during the washing steps. Streptavidin-HRP is added and binds to the biotin-conjugated anti-human IL-10 Ab. Following incubation unbound streptavidin-HRP is removed during the washing steps and substrate solution reactive with HRP is added to the wells. A colored product is formed in proportion to the amount of human IL-10 present in the sample or standard. The reaction is terminated by addition of acid and absorbance is measured at 450 nm.

Procedure:

After reconstitution and preparation of the reagents, the procedure was performed as follows:

1. Microwell strips were washed twice with washing buffer.
2. Hundred µl of standards were added to well.
3. Hundred µl assay buffers were added to the blank wells.
4. Fifty µl of assay buffers were added to sample wells.
5. Fifty µl of samples were added to designated sample wells.
5. The biotin - conjugate was prepared.
6. Fifty µl biotin-conjugates were added to all wells.
7. Microwell strips were covered and incubated for about 2 hours at room temperature (18° to 25°C).

8. Streptavidin-HRP was prepared.
9. Microwell strips were washed 3 times with washing buffer.
10. Hundred µl diluted streptavidin-HRP was added to all wells.
11. Microwell strips were covered and incubated 1 hour at room temperature (18° to 25°C).
12. Microwell strips were washed 3 times with washing buffer.
13. Hundred µl of the TMB substrate solution was added to all wells.
14. The microwell strips were incubated for about 10 minutes at room temperature (18° to 25°C).
15. Hundred µl stop solutions were added to all wells.
16. Microwell reader was blanked and color intensity was measured at 450 nm.

The standard curve was used for the determination of the unknown concentrations in samples assayed at the same time as the standards (Figure 3.2).

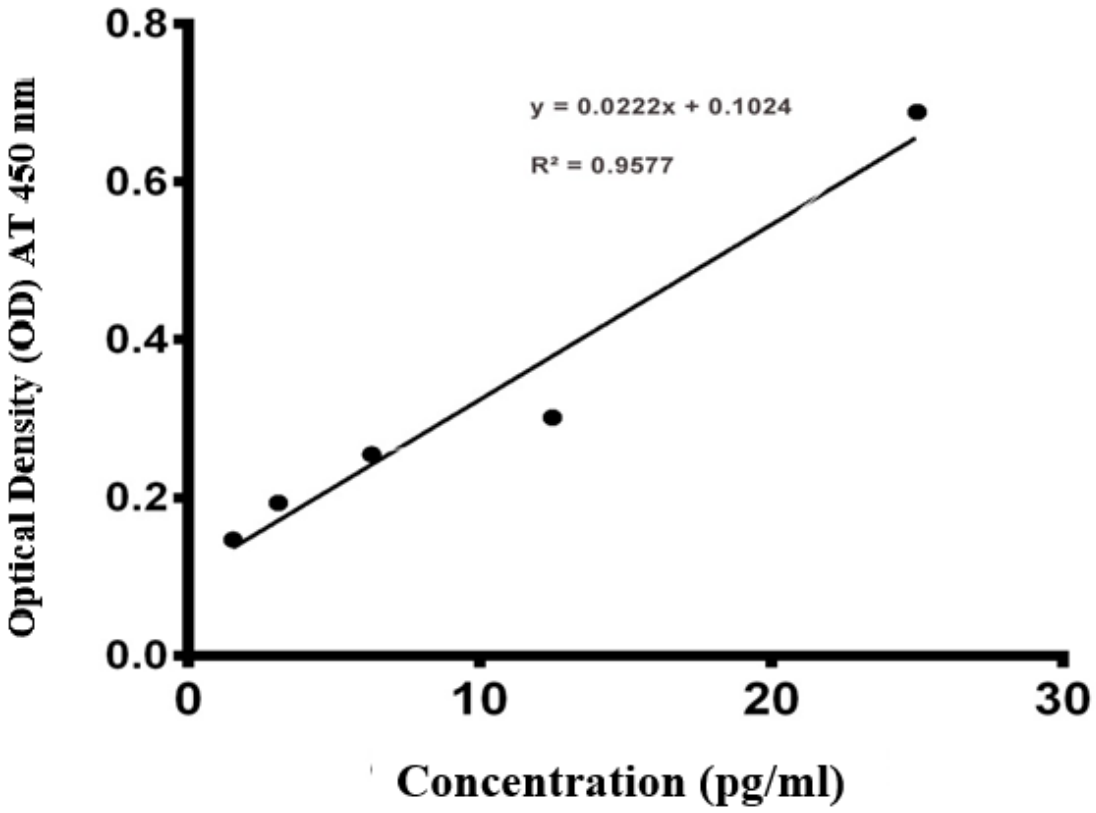


Figure 3.1: Standard curve of IL-10 (pg/ml).

3.2.4.4 Estimation of Serum MDA

Malondialdehyde (MDA) is a byproduct of lipid peroxidation. It would react with thiobarbituric acid (TBA) to produce a complex MDA-TBA. It was determined spectrophotometrically (Lovrić et al., 2008). Serum sample 150 µL and 1ml of 17.5% trichloroacetic acid (TCA) were added into clean centrifuge test tube. One ml of 0.66% TBA was added into the same tube and it was mixed well by vortex 1-2 minutes. The sample was allowed for boiling at 95 ^{o}C in the water bath for about 45 minutes.

After leaving at room temperature 25 °C to cool, 1ml of 70% TCA was added, mixed and centrifuged at 3000 rpm for 15 minutes. The Supernatant, which had a pink color, was read at 532 nm. The optical density changed to concentration (µmole/L) by multiply 42, this number is derived from the following equation:

$$\text{MDA (µmol/L)} = \frac{\textbf{Absorbance at 532nm} \times \textbf{D}}{\textbf{L} * \textbf{E0}}$$

L: light path (1cm)

E_0: Extinction coefficient 1.56×10^5 $M^{-1}.Cm^{-1}$

D: Dilution factor = 1 ml Vol. Used in ref./0.15 =6.7

3.2.4.5 Estimation of NO_2 (representing level of serum nitric oxide)

Griess Reaction was first described in 1879 by Griess. Because of its simplicity, it has been used extensively in the analysis of numerous biological samples including serum, urine, CSF, saliva and cell culture media. In this method, nitrite is first treated with a diazotizing reagent, e.g., sulfanilamide, in acidic media to form a transient diazonium salt. This intermediate is then allowed to react with a coupling reagent, N-naphthyl-ethylenediamine (NNED), to form a stable azo compound. The intense purple color of the product allows nitrite assay with high-sensitivity and can be used to measure nitrite concentration as low as 0.5 mM level. The absorbance of this adduct at 540 nm is linearly proportional to the nitrite concentration in the sample (Sun et al., 2003).

Standard Preparation

Five standard concentrations of sodium nitrite were used with each assay (2.5, 5, 10, 25 and 50 µmol/L) to generate a new equation for each set of samples assayed as shown in (Figure 3.3).

A standard curve was plotted using an optical density value for each standard value versus the concentration of the standards. Then the concentration of each unknown was determined by the interpolation from the standard curve.

Procedure:

1. Samples were deproteinized by mixing 0.5 ml of serum, 10 µL of sodium hydroxide and 300 µL of 0.15 M zinc sulfate, then vortexed by electrical vortex, each sample for one minute. Stand on ice for 15 minutes. Centrifuged for 10 minutes at 3000 rpm.
2. Copper coated cadmium granules were used to convert NO_3 to NO_2, for this step:

   A- Cadmium granules were stored in 0.1 M sulfuric acids were washed by swirling them with distilled water.

   B- A solution of copper sulfate (15 mMol/L in 0.2 mole/L glycine buffer, pH 9.7) was used to coat the granules by mixing them for two minutes.

   C- Cadmium granules were drained and dried over tissue paper was used within 5 minutes.
3. To reduce NO_3 to NO_2:

   A- 0.5 ml of distilled water, standard solutions and deproteinized samples were added to labeled tubes, followed by 0.5 ml of glycine buffer (0.2 mole/L, pH 9.7).

   B- 2 to 3 grams of copper coated cadmium granules were added to the deproteinized samples, the tubes were shaken for 30 minutes on an electric shaker.
4. After the reduction step:-

   A- 0.5 ml of the samples (distilled water, standard solutions and deproteinized samples) were transferred to an appropriately labeled tube followed by the

addition of 0.5 ml of freshly prepared color reagent (Griess reagent). Griess reagent was prepared by mixing an equal volume of 0.1% NNED (1 mg NNED/ml distilled water) and 1 % sulfanilic acid in 5% phosphoric acid (10 mg sulfanilic acid / ml phosphoric acid).

B- Tubes were incubated at room temperature or at 25 ^{o}C in a water bath for color development for 15 minutes.

C- Then absorbance was measured at 543 nm, 1 ml cuvette filled with distilled water as blank.

D- Sodium nitrite was used for preparing the standard curve through plotting absorbance versus concentrations.

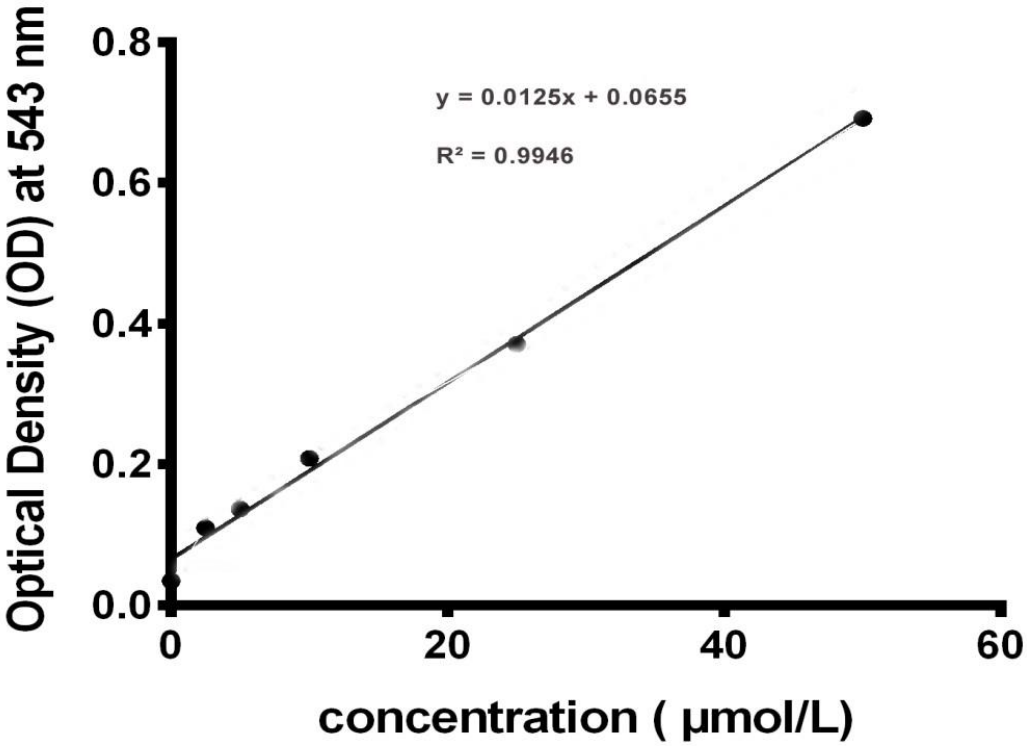


Figure 3.2: Sodium Nitrile Standard Curve

3.3 Statistical Analysis

A Paired Student's t test was used to compare parameters before and after 13 weeks of the KD, using the software package GraphPad Prism version 9.00 for window, GraphPad Software, all data are expressed as mean ± standard error. Normality of data was assessed through the D'Agostino Pearson test, Shapiro-Wilk test and Kolmogorov-Smirnov test. Significance was considered at a value of $p<0.05$.

Unpaired The Student's t test was performed to compare the parameters of the prediabetic group with those of the control group using the software Microsoft Excel 2019 and the package GraphPad Prism version 9.00 for window. All of the data are expressed as the mean along with the standard error. The D'Agostino Pearson test, the Shapiro-Wilk test, and the Kolmogorov-Smirnov test were utilized in order to examine the normality of the data. When the value of p was less than 0.05, significance was assumed to exist. The two-way anova test was used in order to make a comparison between the control group and the prediabetic group regarding the level of blood ketones (beta-hydroxybuty

RESULT

RESULTS

All the participants were maintained on KD program for 13 weeks. During this period, blood ketone level was monitored every week. The mean of weekly ketone body was (1.62± 0.109 mmol/l) and (1.69± 0.124 mmol/l) for control and prediabetes groups respectively (Figure 4.1) and there were consistency in the level of KB during the period of the study. The comparison was performed by two-way ANOVA. P-value<0.05 was regarded as significant.

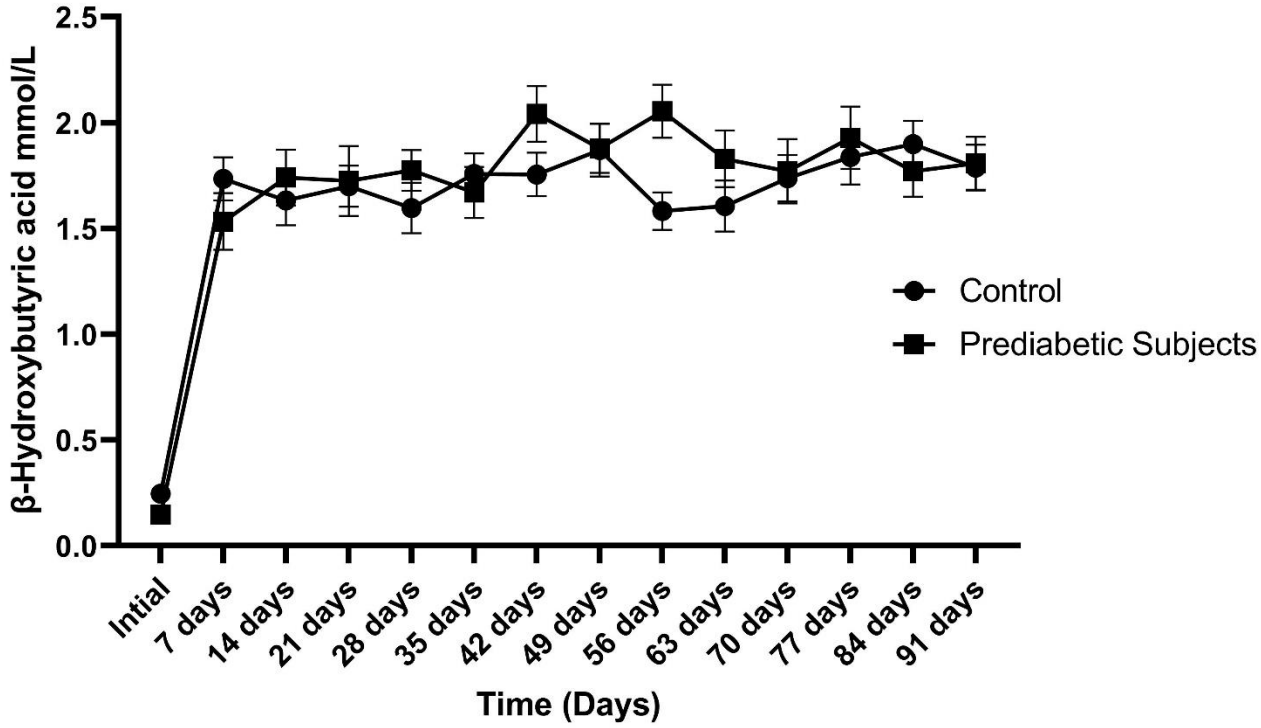


Figure 4.1 Shows the Blood ketone body (beta-hydroxybutyrate) concentration in both healthy and prediabetic individuals.

4.1. Clinical and Laboratory Findings of Healthy Individuals Underwent Ketogenic Diet

Table 4.1: Clinical and Laboratory Findings of Healthy Individuals

Parameter	Normal samples				P Value
	Pre-ketogenic diet		Post-ketogenic diet		
	Mean	SE	Mean	SE	
Alkaline	74.53	2.800	76.88	3.209	0.288
ALT	21.68	1.037	22	0.976	0.727
AST	23.29	0.754	22.74	0.660	0.427
BMI	34.72	0.979	30.55	0.846	<0.0001
BUN	12.10	0.378	14.04	0.542	0.0007
C Peptide	1.626	0.091	1.470	0.083	0.0790
FBS	88.94	1.759	81.29	1.014	0.0003
HOMA-IR	1.183	0.065	1.043	0.060	0.0320
HOMA-S%	94.57	5.885	108.3	7.185	0.0290
HOMA-B%	113.9	6.702	122.4	5.155	0.200
LDL	115.6	3.332	120.6	4.206	0.06
HDL	38.70	1.523	38.34	1.603	0.71
VLDL	28.71	2.135	36.32	1.924	<0.0001
TG	143.6	10.67	181.6	9.620	<0.0001
Cholesterol	174.4	5.858	187.8	8.273	0.0328
Creatinine	0.867	0.018	1.044	0.043	0.0004
Urea	31.06	0.971	33.79	0.971	0.0034
Uric Acid	6.013	0.106	7.515	0.303	<0.0001
NO	8.893	0.945	12.39	1.662	0.1195
MDA	4.569	0.300	5.020	0.222	0.0834
IL 10	1.452	0.328	3.380	0.601	0.0164

4.1.1 Body Mass Index (BMI)

The control group participants who were recruited into this study had an average age (34.74 ± 1.511 years). About 82% (n=28) of them were male and 18% (n=6) of them were female. Concerning BMI, there was a significant (P-value ≤0.0001) decrease in BMI in post ketogenic diet (30.55 ± 0.846), compared to pre ketogenic diet (34.72 ± 0.979) (Figure 4.2).

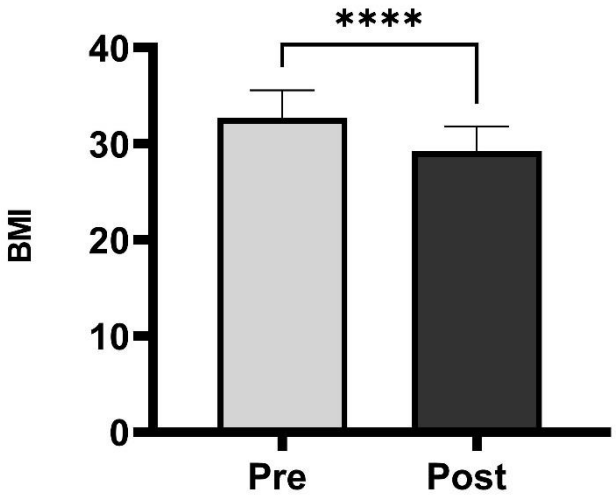


Figure 4.2: Comparison of BMI between pre and post KD in Control group; BMI ,Body Mass Index; Comparison was done by paired t-test.

4.1.2 Fasting Blood Sugar (FBS) Level

A significant difference (P-value =0.0003) in FBS was found between the pre KD (88.94± 1.759 mg/dl) and the post KD in (81.29± 1.014 mg/dl) (Figure 4.3).

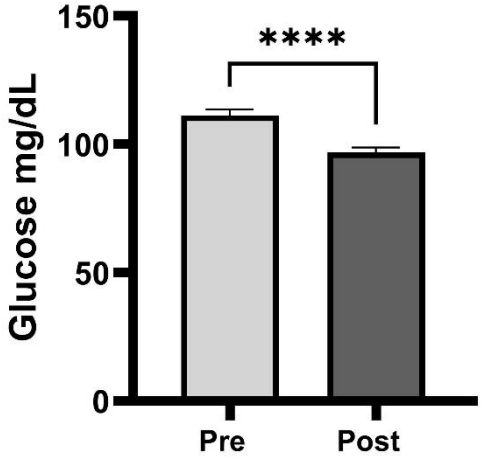


Figure 4.3: Comparison of Fasting Blood sugar between pre and post KD in Control group ;Comparison was done by paired t-test.

4.1.3 C Peptide and HOMA Measurements

The mean of C peptide in the pre KD was (1.626±0.090ng/mL). It was significantly (P-value =0.0790) reduced to (1.470±0.083 ng/mL) after the 13 weeks of feeding. The insulin resistance, HOMA-IR, was significantly decreased between pre ketogenic and post ketogenic (from 1.183± 0.065 to 1.043± 0.060 P-value =0.032). Moreover, the result of insulin sensitivity, expressed by HOMA-S%, was significantly (P value =0.029) increased in post KD (108.3±7.185) as compared to pre ketogenic group (94.57±5.885). The assessment of beta-cell function, HOMA-B%, showed a non-significant change between pre and post ketogenic groups (from 113.9± 6.702 to 122.4± 5.155 P-value =0.2) (Figure 4.4).

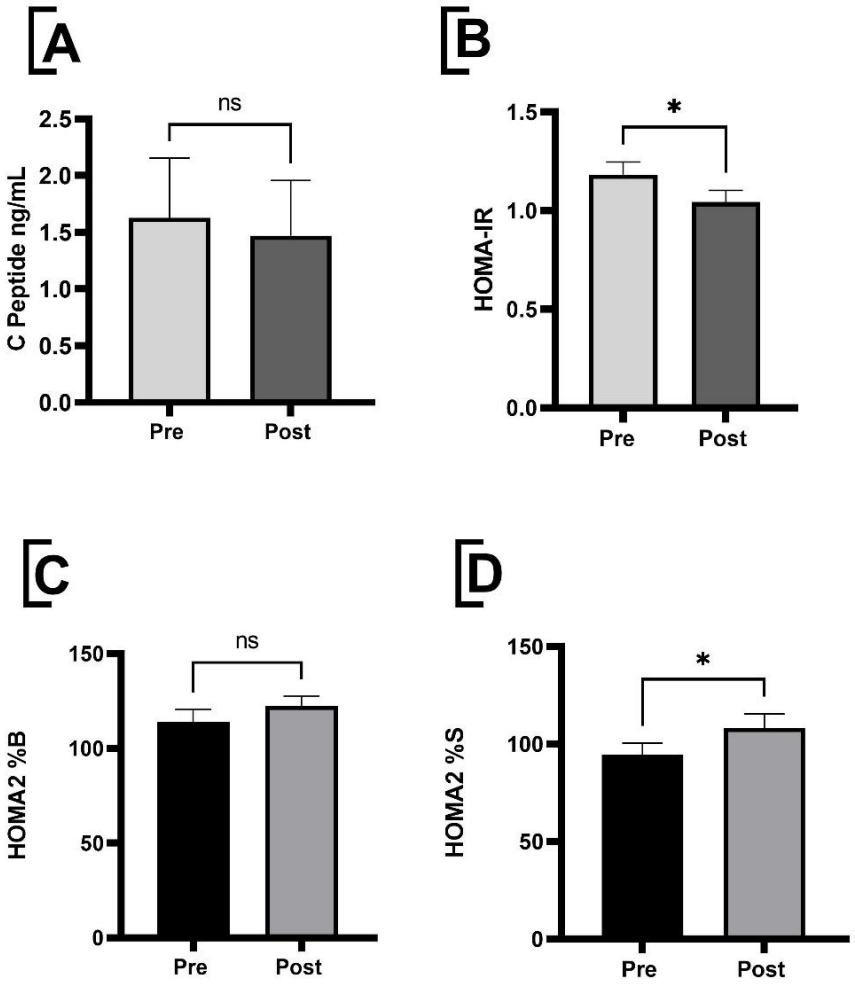


Figure 4.4: Comparison of different variables between pre and post KD in the control group. A-C Peptide B- HOMA IR C- HOMA %B D- HOMA %S; Comparison was done by paired t-test. HOMA IR, homeostasis model assessment-estimated insulin resistance; HOMA %B , homeostasis model assessment-estimated β-cell function; HOMA %S, homeostasis model assessment-estimated cell sensitivity function.

4.1.4 Serum Lipid Profiles Level

Total serum cholesterol was elevated significantly (from 174.4± 5.858 to 187.8± 8.273 mg/dl P-value= 0.0328), while TG was significantly higher in the

post KD compared to the pre KD (from 143.6±10.67 to 181.6 ± 9.62 mg/dl P-value<0.0001).On the other hand, the KD had no significant effect on serum level of LDL(from 115.6± 3.332 to 120.6± 4.206 mg/dl P-value= 0.06) ,and HDL (from 38.70± 1.523 to 38.34± 1.603 mg/dl P-value= 0.71).While, VLDL shows significant difference (from 28.71± 2.135 to 36.32± 1.924 mg/dl P-value <0.0001) between pre and port KD (Figure 4.5).

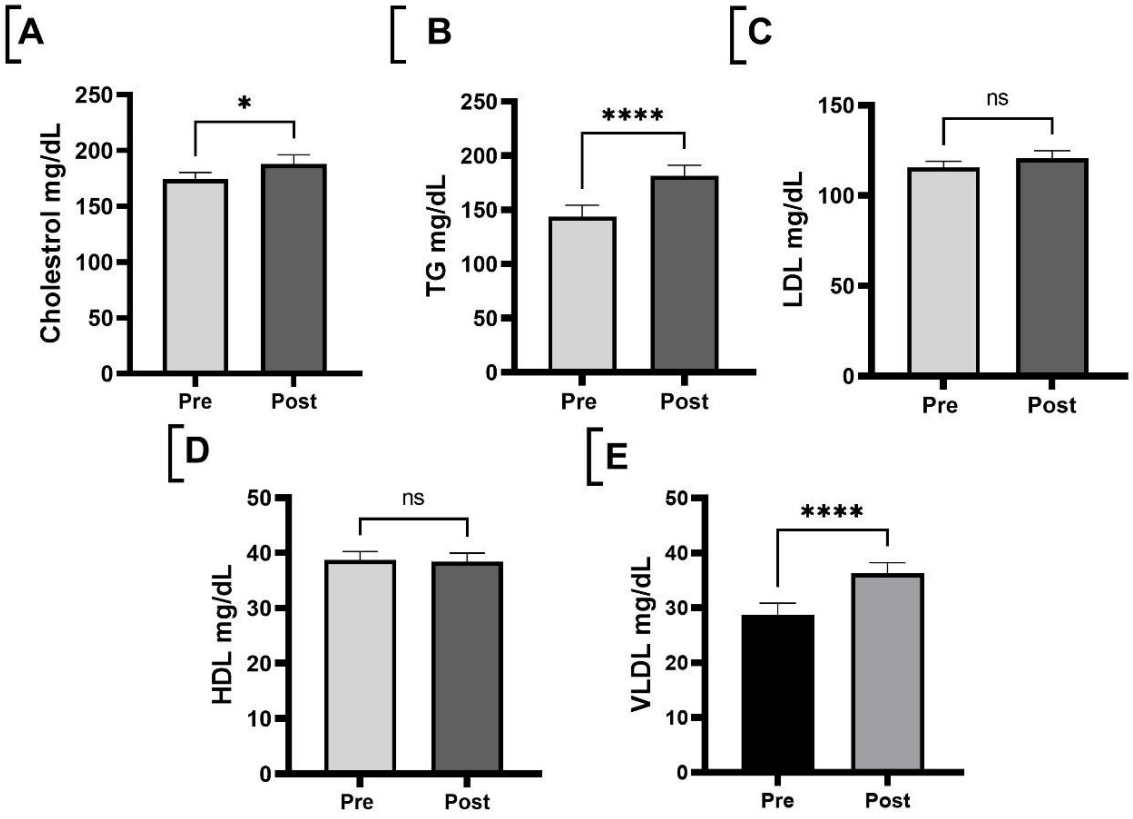


Figure 4.5: Comparison of different variables between pre and post KD in the control group. A-cholesterol B- TG C-LDL D-HDL E-VLDL; The comparison was done by paired t-test.TG, triglycerides; LDL, low-density lipoprotein; HDL, High-density lipoprotein; VLDL, Very low-density lipoprotein; KD, Ketogenic diet.

4.1.5 Kidney and Liver Function Parameters

Non-significant changes were found for ALP (from 74.53± 2.8 to 76.88± 3.209 IU/L P-value= 0.288), AST (from 23.29± 0.754 to 22.74± 0.66 IU/L P-value= 0.427) and ALT (from 21.68± 1.037 to 22 ± 0.976 IU/L P-value= 0.727) between pre and post ketogenic diets (Figure 4.6).

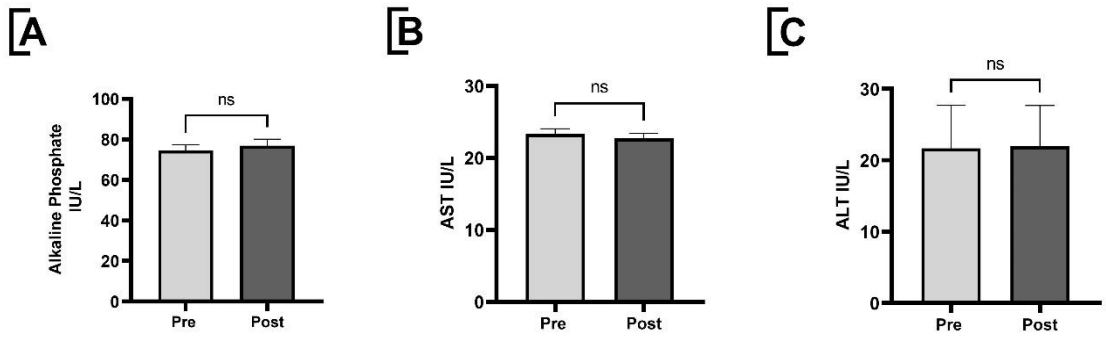


Figure 4.6: Comparison of different variables between pre and post KD in the control group. A-alkaline phosphatase B- AST C-ALT The comparison was done by paired t-test; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

While the kidney function tests (KFTs) including creatinine, Urea, Uric Acid, and BUN parameters were increased significantly in post ketogenic diet as compared to corresponding parameters in pre ketogenic diets. The creatinine value increased significantly from (0.867 ± 0.018 to 1.044± 0.043 mg/dl P-value= 0.0004). Likewise, Urea level increased significantly from (31.06± 0.971 to 33.79± 0.971 mg/dl P-value= 0.0034). However, the Uric Acid level in control group increased from (6.013± 0.106 to 7.515± 0.303 mg/dl P-value <0.0001). Furthermore, BUN increased from (12.10± 0.378 to 14.04 ± 0.542 mg/dl P-value =0.0007).

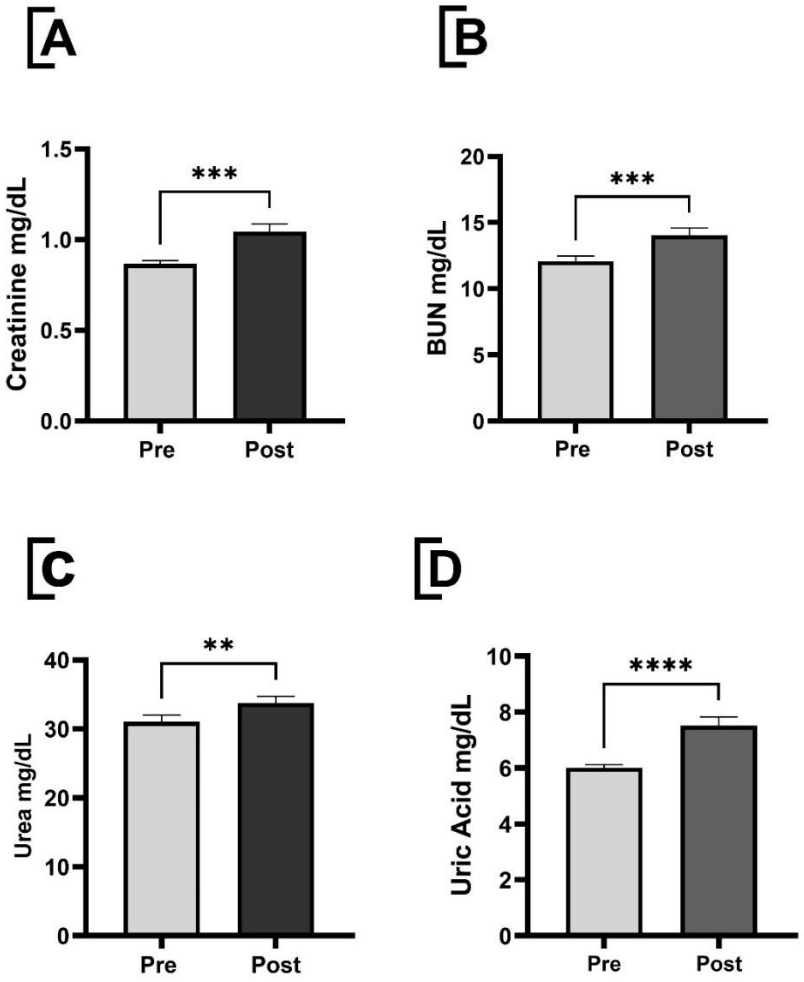


Figure 4.7: Comparison of different variables between pre and post KD in the control group. A-Creatinine B- BUN C-Urea D-Uric Acid; The comparison was done by paired t-test.; BUN, blood urea nitrogen.

4.1.6 Oxidative Stress Parameters

Participants in the control group increased their NO level to (12.39±1.662 µmol/L) non significantly (P-value =0.120) compared to pre KD diet (8.893±0.945 µmol/L). However, the effect of KD was also non-significant for MDA (from 4.569 ± 0.3 to 5.02 ± 0.222 µmol/L P-value =0.0834) (Figure 4.8).

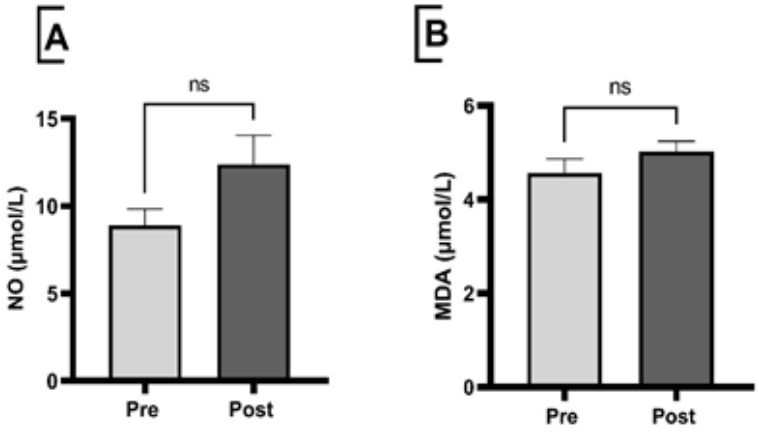


Figure 4.8: Comparison of different variables between pre and post KD in the control group. A- NO B- MDA ;Comparison was done by paired t-test. NO, Nitric Oxide; MDA, Malonaldehyde.

4.1.7 Serum level of IL 10

KD has a significant impact on the level of serum IL10 (P-value =0.0164), the amount of serum IL10 increases non significantly (from 1.452± 0.328 to 3.380± 0.601 pg/ml) (Figure 4.9).

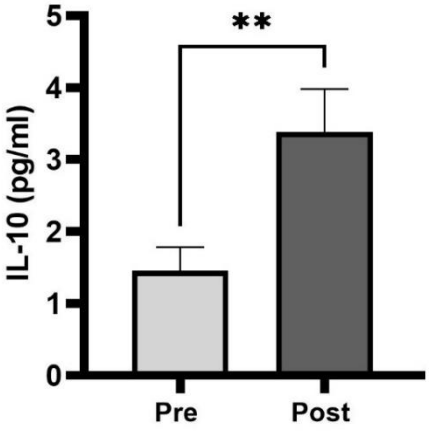


Figure 4.9: Comparison of level of IL10 between pre and post KD in the control group.; Comparison was done by paired t-test. IL10, Interleukin 10.

4.2 Clinical Profile of Prediabetes Participants who were Undergoing a Ketogenic Diet (The comparison between pre and post KD program)

Table 4.2: Clinical and Laboratory Findings of Prediabetes Participant**s**

Parameter	Prediabetes samples				P Value
	Pre-ketogenic diet		Post-ketogenic diet		
	Mean	SE	Mean	SE	
Alkaline	73.05	3.708	74.56	3.338	0.2768
ALT	21.38	1.176	22.13	1.026	0.3865
AST	22.75	1.232	22.04	0.829	0.387
BMI	32.69	0.650	29.23	0.578	<0.0001
BUN	12.03	0.503	14.48	0.718	<0.0001
C Peptide	3.300	0.172	2.403	0.154	<0.0001
FBS	111.3	2.408	96.96	1.896	<0.0001
HOMA-IR	2.580	0.141	1.802	0.115	<0.0001
HOMA-S%	40.89	1.826	62.20	4.988	<0.0001
HOMA-B%	122.6	5.956	124.5	7.789	0.8350
LDL	131.1	8.798	124.3	9.909	0.012
HDL	42.87	2.014	45.65	1.764	0.0130
VLDL	28.71	2.326	32.06	2.823	0.0008
TG	143.5	11.63	176.8	15.40	0.0002
Cholesterol	189.9	7.461	198.8	9.031	0.146
Creatinine	0.706	0.024	0.853	0.03	<0.0001
Urea	31.42	1.536	34.42	1.511	<0.0001
Uric Acid	5.938	0.194	7.038	0.25	0.0003
NO	4.314	0.958	6.614	1.220	0.0140
MDA	4.383	0.376	5.178	0.285	0.0409
IL 10	1.684	0.315	3.657	0.96	0.0722

4.2.1 BMI

The Prediabetes participants who were recruited into this study had an average age (35.1 ± 1.354 years); 83% (n=20) of them were male; 17% (n=4) of them were female. Concerning BMI, there was a significant (P-value ≤0.0001) decrease in BMI in post ketogenic diet (29.23 ± 0.578), compared to pre ketogenic diet (32.69 ± 0.65) (Figure 4.10).

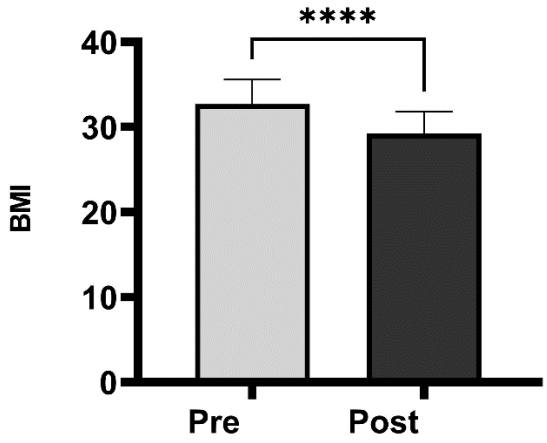


Figure 4.10: Comparison of BMI between pre and post KD in Prediabetic group. Comparison was done by paired t-test.; BMI, body mass index.

4.2.2 Level of Serum FBS

A significant difference (P-value ≤0.0001) in FBS was found between the pre ketogenic diet (111.3± 2.408 mg/dl) and the post ketogenic diet in (96.96± 1.896 mg/dl) (Figure 4.11).

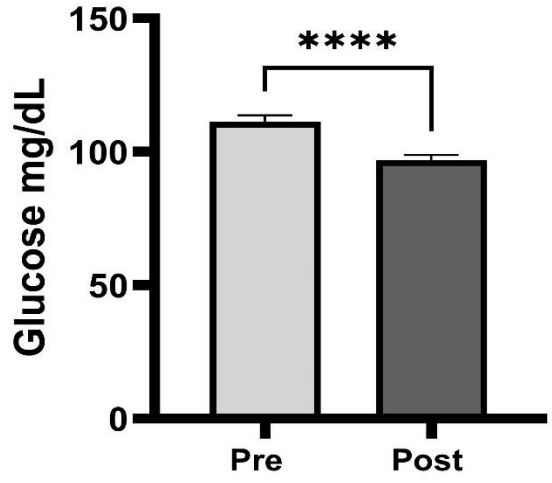


Figure 4.11: Comparison of FBS between pre and post KD in Prediabetic group. Comparison was done by paired t-test.; FBS, Fasting Blood Sugar.

4.2.3 C Peptide and HOMA Measurement

The KD has a highly significant effect on serum C-peptide (P-value =<0.0001) value in blood. The mean of C-peptide in the prediabetes group was (3.3 ±0.172 ng/mL), and it lowered significantly to (2.403±0. 015 ng/mL) after 13 weeks of KD. Moreover, C peptide and glucose significantly reduced the HOMA parameters. HOMA-IR (Insulin Resistance) and HOMA- S% (Insulin Sensitivity) were altered significantly ((from 2.580 ± 0.141 to 1.802± 0.115 P-value <0.0001), (from 40.89± 1.826 to 62.20± 4.988 P-value <0.0001) respectively)in post KD for prediabetes group, Furthermore, the HOMA-B%, pancreatic beta-cell function indicator, was non-significantly changed by KD (from 122.6± 5.956 to 124.5± 7.789 P-value =0.835) (Figure 4.12).

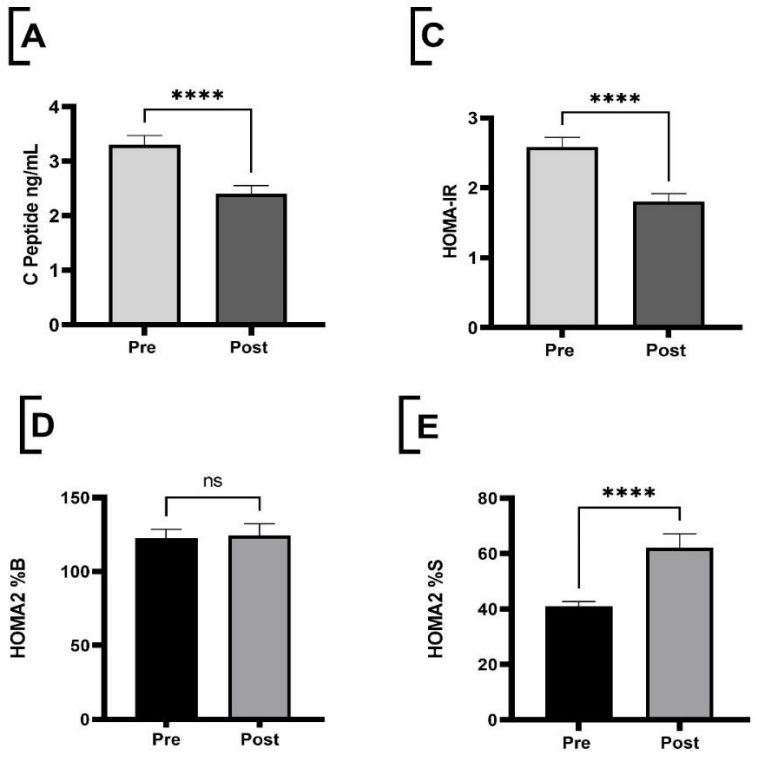


Figure 4.12: Comparison of different variables between pre and post KD in the prediabetic group. A-C Peptide B- HOMA IR C- HOMA %B D- HOMA %S; The comparison was done by paired t-test.; HOMA IR, homeostasis model

assessment-estimated insulin resistance; HOMA %B , homeostasis model assessment-estimated β-cell function; HOMA %S, homeostasis model assessment-estimated cell sensitivity function.

4.2.4 Serum Lipid Profiles Level

Compared to the pre-ketogenic diet, the effect of KD on serum cholesterol levels was non-significant (from 189.9± 7.461to 198.8± 9.031 mg/dl P-value= 0.146), While TG levels were considerably higher in the post-ketogenic diet (from 143.5± 11.63 to 176.8± 15.40 mg/dl P-value 0.0002,). In addition, serum LDL level lowered (from 131.1± 8.798 to 124.3± 9.909 mg/dl P-value= 0.012) and HDL elevated (from 42.87± 2.014 to 45.65± 1.764 mg/dl P-value= 0.013). VLDL lipid profiles did alter significantly (from 28.70± 2.326 to 32.06± 2.823 mg/dl P-value= 0.0008) between before and post-ketogenic diets, (Figure 4.13).

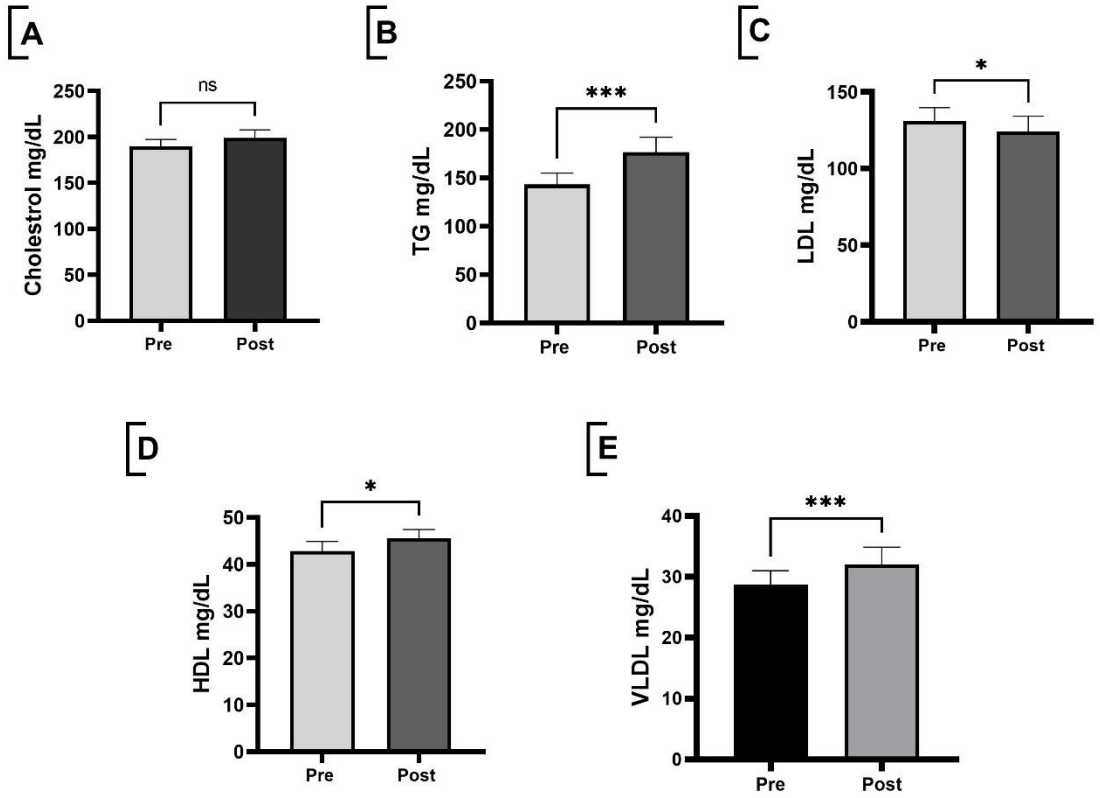


Figure 4.13: Comparison of different variables between pre and post KD in the prediabetes group. A-Cholestrol B-TG C-LDL D-HDL E-VLDL;The comparison was done by paired t-test; TG, Triglyceride; LDL, low-density lipoprotein; HDL, High-density lipoprotein; VLDL, Very low-density lipoprotein; KD, Ketogenic diet.

4.2.5 Kidney and Liver Test Parameters

As can be seen in (Figure 4.14), non-significant change seen between pre and post ketogenic diets for ALP (from 73.05 ± 3.708 to 74.56 ± 3.338 IU/L P-value= 0.277), AST (from 22.75 ± 1.232 to 22.04 ± 0.829 IU/L P-value= 0.387) and ALT (from 21.38 ± 1.176 to 22.13 ± 1.026 IU/L P-value= 0.388).

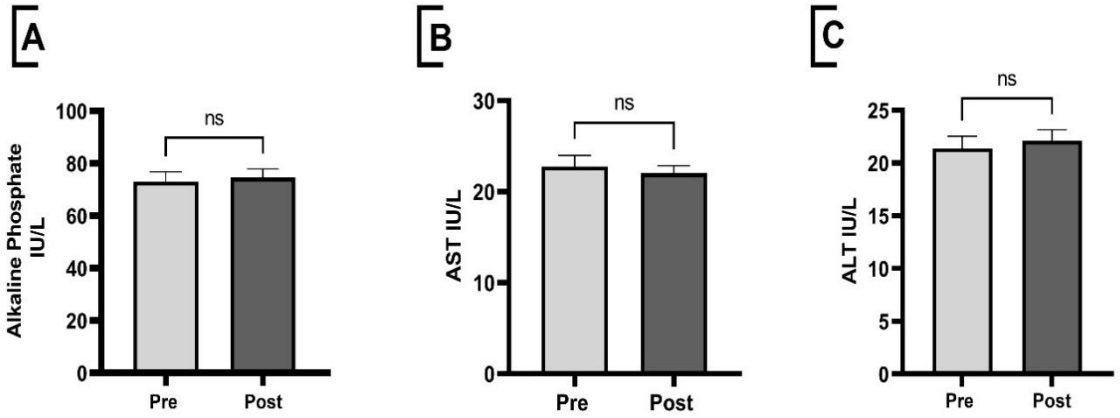


Figure 4.14: Comparison of different variables between pre and post KD in Prediabetes group. A-alkaline phosphatase B- AST C-ALT; Comparison was done by paired t-test; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

While, The serum Creatinine ,Urea, Uric Acid, and BUN parameters level was increase significantly ((from 0.706± 0.024 to 0.853 ± 0.03 mg/dl P-value <0.0001), (from 31.42± 1.536 to 34.42 ± 1.511mg/dl P-value <0.0001), (from 5.938± 0.194 to 7.038± 0.25 mg/dl P-value =0.0003),and (from 12.03± 0.503 to 14.48± 0.718 mg/dl P-value <0.0001) respectively) (Figure 4.15).

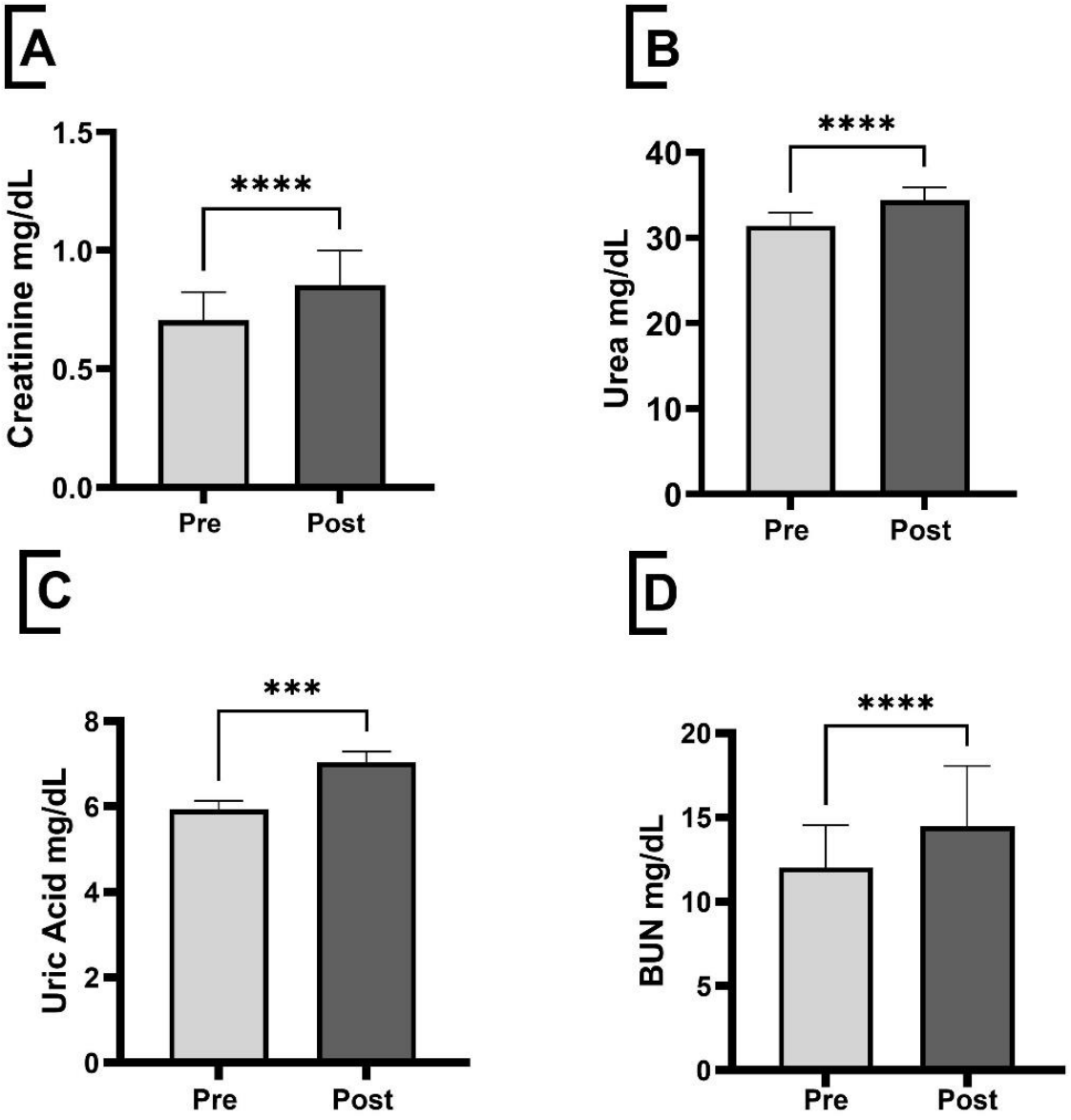


Figure 4.15 Comparison of different variables between pre and post KD in Prediabetes group. A -Creatinine B-Urea C-Uric Acid D- BUN. Comparison was done by paired t-test; BUN, blood urea nitrogen.

4.2.6 Oxidative Stress Parameters

In the Prediabetes group, the oxidative stress parameters were significantly increased for both NO (from 4.314±0.958 to 6.614±1.22 µmol/L P-value =0.014) and MDA (from 4.383 ±0.376 to 5.178 ±0.285 µmol/L P-value =0.0409) (Figure 4.16).

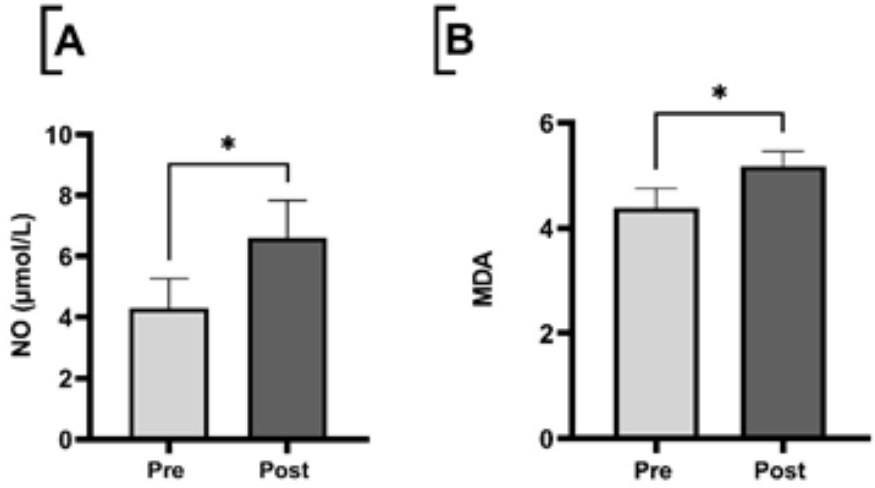


Figure 4.16: Comparison of different variables between pre and post KD in the Prediabetes group; A- NO B- MDA ;Comparison was done by paired t-test; NO, Nitric Oxide; MDA, Malonaldehyde.

4.2.7 Serum level of IL 10

The IL10 level in serum of Prediabetes Group individuals was increased non significantly (from 1.684± 0.315to 3.657± 0.96 pg/ml P-value =0.0722) (Figure 4.17).

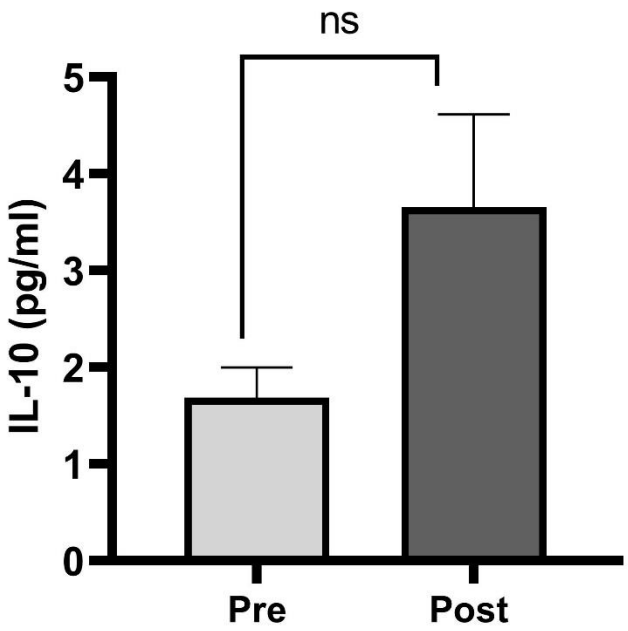


Figure 4.17: Comparison of level of IL10 between pre and post KD in the Prediabetes: Comparison was done by paired t-test; IL10, Interleukin 10.

4.3 Comparison between Mean Differences of the Variable in the Control and Prediabetes Groups.

4.3.1 BMI

The mean difference in BMI in the control group was (-3.959 ± 0.317), which was significantly (P-value =0.031) higher than in the prediabetes group (-2.883 ± 0.361) (Figure 4.18).

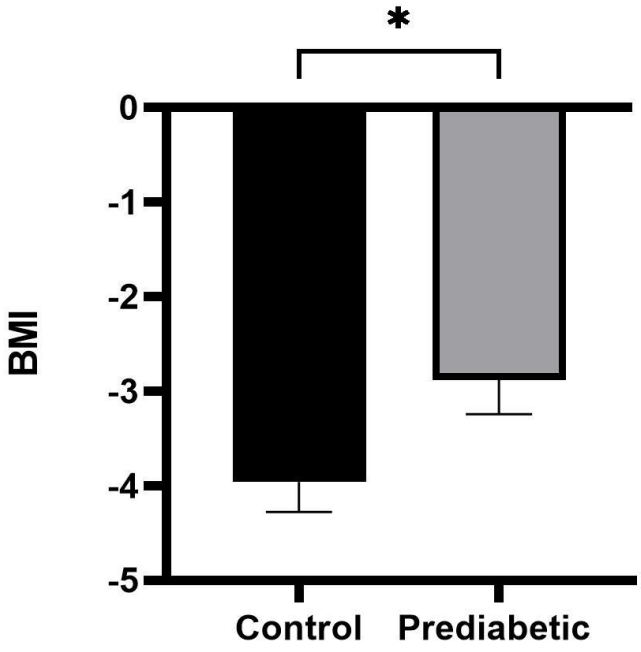


Figure 4.18: Comparison of the mean difference between BMI of the control group and prediabetes group; Comparison was done by unpaired t-test.

4.3.2 Mean Difference of FBS

The comparison of mean deference of FBS of the control (-7.647 ± 1.866 mg/dl) and prediabetes (-13.63 ± 2.836 mg/dl)groups showed no significant difference (P-value =0.0716) (Figure 4.19).

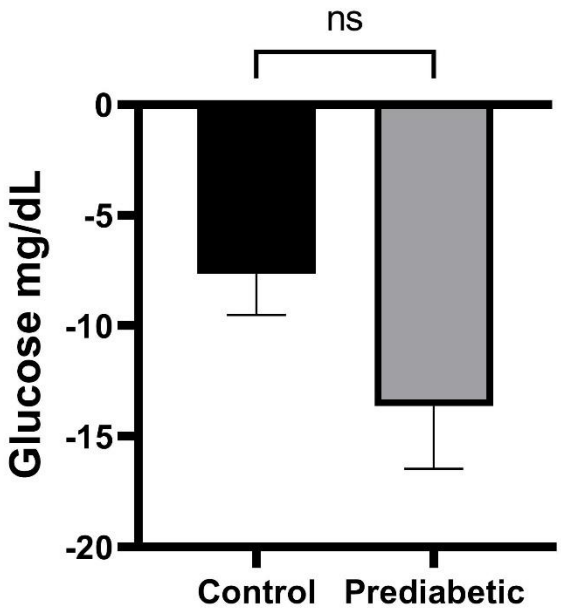


Figure 4.19: Comparison of the mean difference between FBS of the control group and prediabetes group; Comparison was done by unpaired t-test.

4.3.3 Mean Difference of C Peptide and HOMA Levels

The mean difference of C-peptide was significantly different (P-value <0.0001). The mean difference of the control group was (-0.156 ± 0.860 ng/mL) as compared to (-0.980 ± 0.170 ng/mL) in the prediabetes group. A significant difference was also seen in HOMA IR (P-value <0.0001), result shows that the mean difference of HOMA IR of prediabetes group (-0.803± 0.136) decreased compared to the control group (-0.141± 0.063).While, there was no significant difference (P-value = 0.427) for mean difference of HOMA S%, the cell sensitivity of prediabetes group (20.02± 3.540) was increased non significantly compare to control group (13.73± 6.013).

Concerning, the HOMA B% for beta cell function, the result shows that the mean difference of control group was (8.509± 6.509) and the mean difference of prediabetes group was (6.988 ± 9.514) with no significant difference (P-value = 0.892) (Figure 4.20).

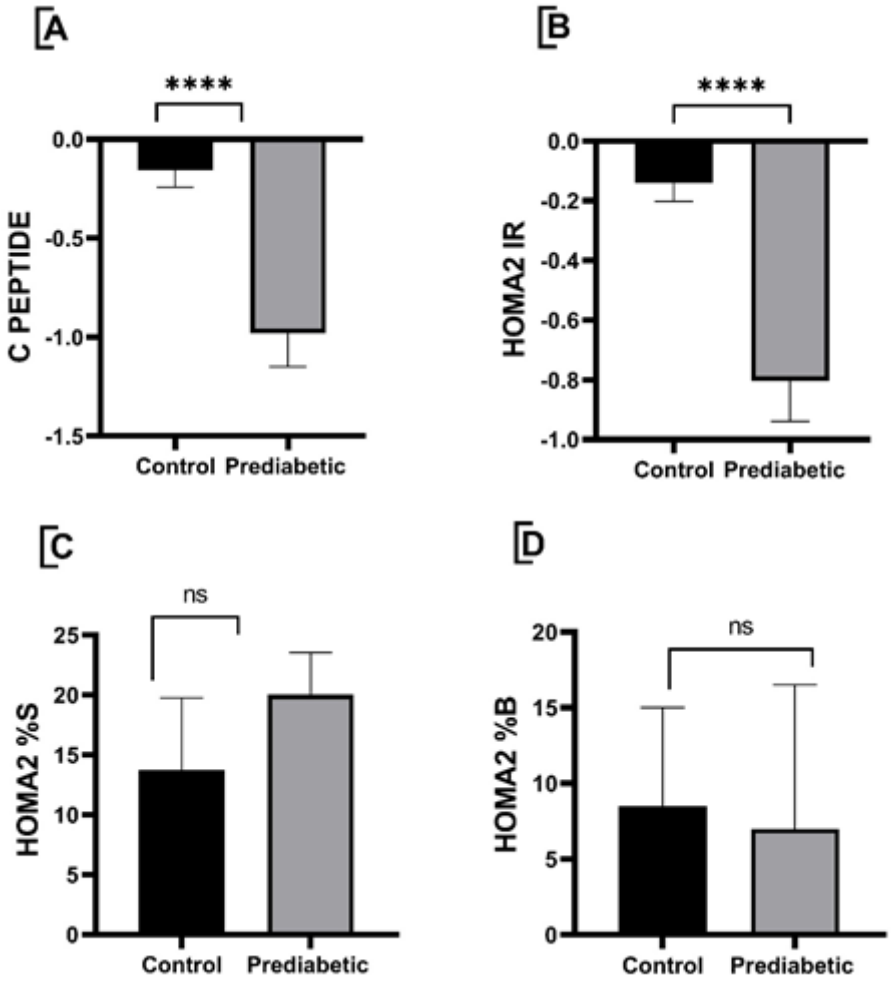


Figure 4.20: Comparison of different variables between the control group and prediabetic. A-C Peptide B- HOMA IR C- HOMA %S D- HOMA %B. The comparison was done by unpaired t-test; HOMA IR, homeostasis model assessment-estimated insulin resistance; HOMA %B, homeostasis model assessment-estimated β-cell function; HOMA %S, homeostasis model assessment-estimated cell sensitivity function.

4.3.4 Serum Lipid Profiles

There was no significant difference in the mean difference of serum cholesterol (P-value =0.592) in between control (13.41± 5.168 mg/dl) and prediabetes group (8.875± 6.859 mg/dl). Furthermore, The mean difference of TG of control group was (38.06± 8.209 mg/dl) compare to prediabetes group (28.57± 6.297 mg/dl) with no significant difference (P-value = 0.403).The mean difference was significant for LDL; the mean difference of LDL for the control group (4.944 ± 2.535 mg/dl) was higher than the prediabetes group (-6.806 ± 3.032 mg/dl). However, the mean difference of HDL in the control group (-1.366 ± 1.005 mg/dl) was lower than in the prediabetes group (2.096 ± 1 mg/dl). and the mean difference VLDL (Control 7.612 ± 1.642 mg/dl vs Prediabetic 6.65 ± 1.527 mg/dl; P-value =0.681) (Figure 4.21).

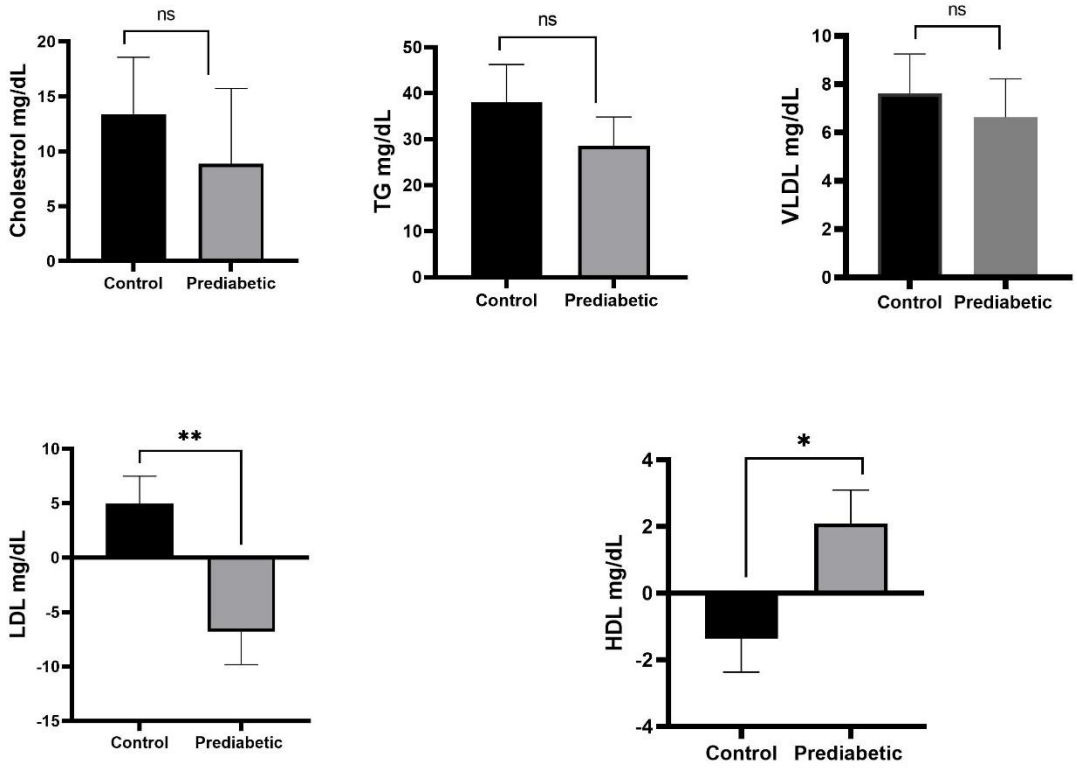


Figure 4.21: Comparison of mean difference between variables of control group and prediabetic group A-Cholesterol B-TG C-VLDL D-LDL E-HDL Comparison was done by unpaired t-test; P-value<0.05 was regarded as significant; TG, triglycerides; LDL, low density lipoprotein; HDL, High density lipoprotein; VLDL, Very low density lipoprotein.

4.3.5 Kidney and Liver Test Parameters

Regarding liver parameters, the result show no significant difference between mean difference of ALP(Control 2.671 ± 1.754 IU/L vs Prediabetic 2.3 ± 0.921 IU/L; P-value= 0.868),AST(Control -0.505 ± 0.677 IU/L vs Prediabetic -0.708 ± 0.804 IU/L; P-value= 0.848), and ALT (Control -0.466 ± 0.859 IU/L vs Prediabetic 0.859 ± 0.85 IU/L; P-value= 0.822) (Figure 4.22).

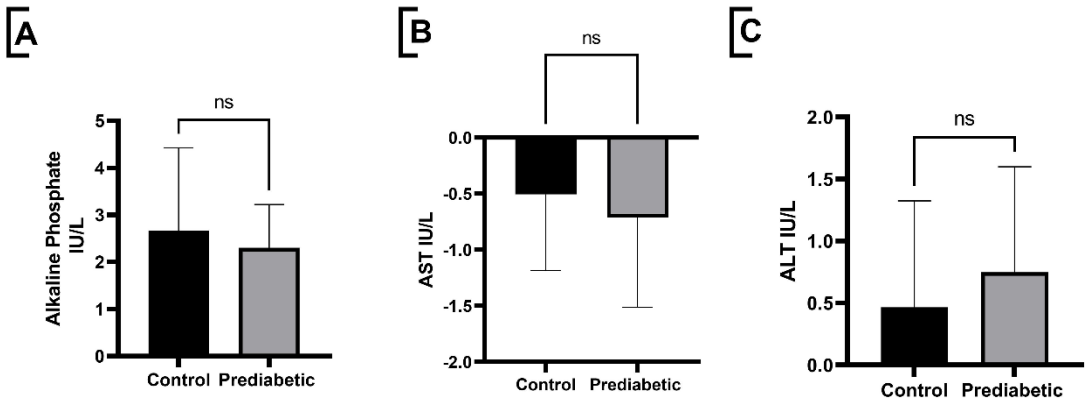


Figure 4.22: Comparison of different variables between the control group and prediabetes; A-alkaline phosphatase B- AST C-ALT. Comparison was done by unpaired t-test.; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

The mean difference of Creatinine between control and prediabetes group was non significant (Control 0.177 ± 0.043 mg/dl vs Prediabetic 0.147 ± 0.022 mg/dl; P-value= 0.576).Likewise for mean difference of Urea (Control 2.435 ± 0.653 mg/dl vs Prediabetic 3 ± 0.634 mg/dl; P-value= 0.580),Uric acid (Control 1.812 ± 0.319 mg/dl vs Prediabetic 1.017 ± 0.211 mg/dl; P-value= 0.063),and BUN (Control 1.946 ± 0.537 mg/dl vs Prediabetic 2.446 ± 0.412 mg/dl; P-value= 0.494) (Figure 4.23).

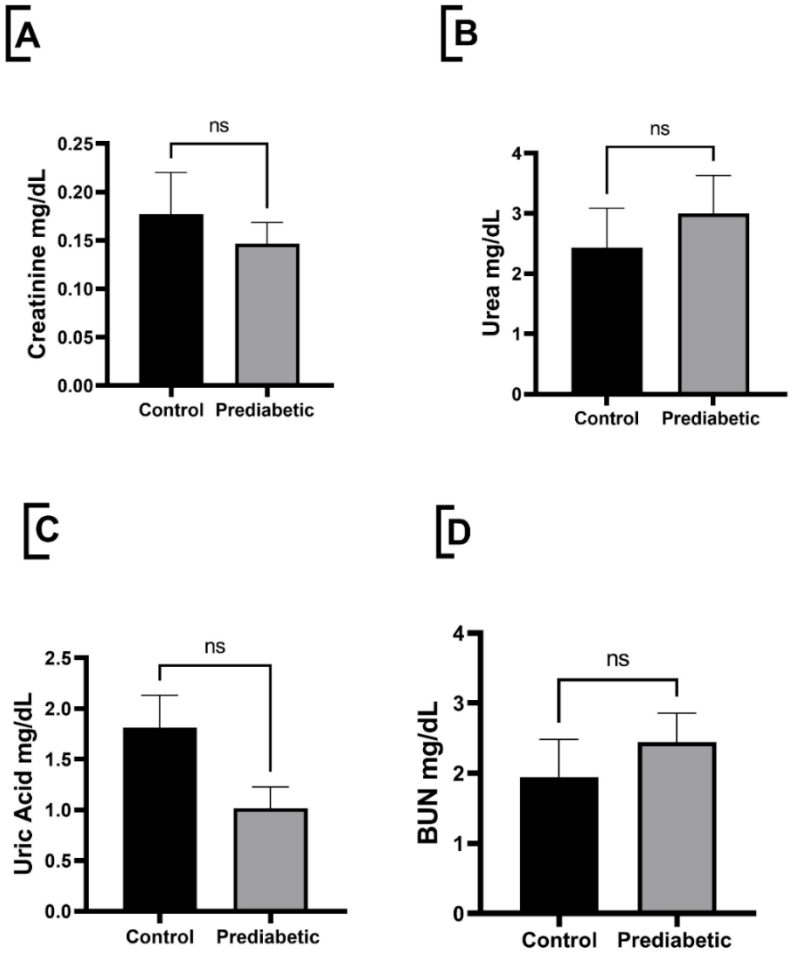


Figure 4.23: Comparison of different variables between the control group and prediabetes. A- Creatinine B-Urea C-Uric Acid D- BUN; Comparison was done by unpaired t-test.; BUN, blood urea nitrogen.

4.3.6 Oxidative Stress Parameters

Concerning Oxidative Stress, there were no significant differences between the mean difference of oxidative stress parameters NO (Control 2.671 ± 1.754mg/dl vs Prediabetic 2.3 ± 0.921 mg/dl; P-value =0.868) and MDA (Control 0.451 ± 0.366 mg/dl vs Prediabetic 0.796 ± 0.367 mg/dl; P-value =0.521) (Figure 4.23).

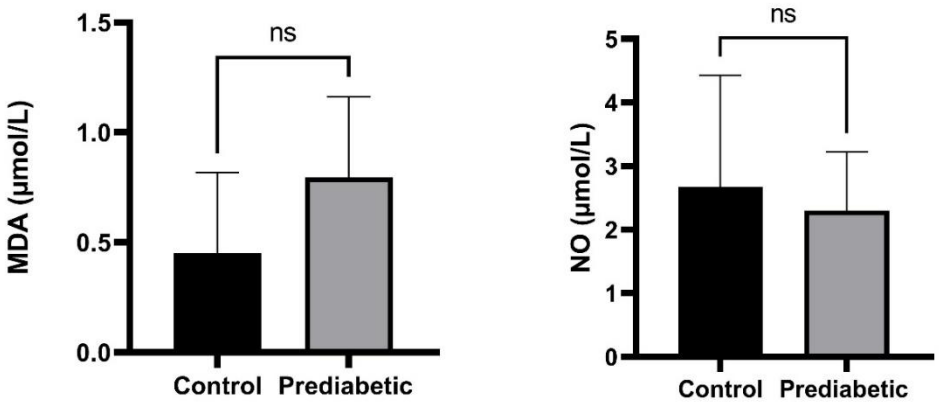


Figure 4.24 Comparison of different variables between the control group and prediabetic. A- NO B- MDA IR C- IL-10-. The comparison was done by unpaired t-test.; NO, Nitric Oxide; MDA, Malonaldehyde.

4.3.7 Mean Difference of Serum IL 10

The mean difference of IL10 shows non-significant different (Control 0.96 ± 0.277mg/dl vs Prediabetic 0.729 ± 0.625 mg/dl; P-value =0. 0.742), (Figure 4.23).

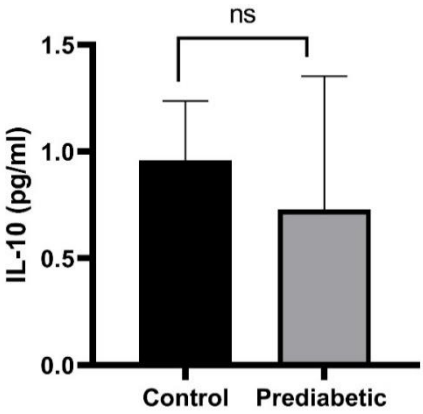


Figure 4.25 Comparison of IL10 between the control group and prediabetes; The comparison was done by unpaired t-test; IL10, Interleukin 10.

Discussion

5-Discussion

The prevalence of obesity has increased worldwide in the past ~50 years, reaching pandemic levels. Obesity represents a major health challenge because it substantially increases the risk of diseases such as type 2 diabetes mellitus, fatty liver disease, hypertension, myocardial infarction, stroke, dementia, osteoarthritis, obstructive sleep apnoea and several cancers, thereby contributing to a decline in both quality of life and life expectancy. Obesity is also associated with unemployment, social disadvantages and reduced socio-economic productivity, thus increasingly creating an economic burden (Blüher, 2019). Nutritional ketosis is a natural phenomenon when man reduces carb intake remarkably. Many researches shows that the KD is very effective for weight reduction in a manner that it can be used as a therapeutically use for any rare disease (Gershuni et al., 2018) . Still, the evidence of the benefit or impact of KD on the human body is limited, especially for the long term effect on prediabetes individuals.

5.1 Effect of KD on BMI and Body Weight

This study demonstrates weight reduction and BMI of both control and prediabetes groups, which can be explained by the deficient carbohydrate intake, which turns the body to a nutritional ketosis state. Thus, the body's energy supply turns from glucose to ketone bodies, which promote the secretion of glucagon hormones that enhance the lipolysis, gluconeogenesis and inhibit fat synthesis. The process of gluconeogenesis is converting fat from adipose tissue to glucose that is needed to maintain blood glucose levels, which is the catabolic process, and the amount of blood ketone bodies are excreted through urine (Urbain and Bertz, 2016) Furthermore, nutritional ketosis suppresses the appetite (Nymo et al., 2017). This because the fat and proteins require long

period for digestion Therefore, it stay in digestive system longer time than carbohydrate.as well the ketogenic foods enhance secretion of an hormone which released by gut called cholecystokinin which is very strong in suppression of the appetite.(Bekkouche et al., 2014).Concluded that ketone bodies contribute to the satiating effect by inhibiting the appetite centre in brain (Deemer et al., 2020).Furthermore, proteins have a greater anorexigenic effect than carbs. This could be related to enhanced sensitivity of the central nervous system to the appetite suppressant hormone leptin and lower postprandial plasma concentrations of the hunger-stimulating hormone ghrelin. All of this can be summed that ketosis suppresses hunger, which, in conjunction with the changes in appetite-regulating hormone levels, may promote weight loss(Deemer et al., 2020, Paoli et al., 2013a).

5.2 Effect of KD on Glycemic Control and Insulin Resistance

The massive decrease in FBS was seen in the prediabetes and control groups. This improvement was due to significantly restricting the consumption of carbohydrates, which regulates the degradation of stored glycogen and may also reduce intestinal sugar absorption (Yancy Jr et al., 2003, Bolla et al., 2019). The current result was parallel with other findings of Partsalaki et al. (2012), Saslow et al. (2017), Myette-Côté et al. (2018) on the other hand the improvement in glycemic control could sometimes be due to an altered gene regulation by transcription factors such as NF- κ B or PPAR γ are based (Tilg and Moschen, 2008). PPARγ activation in mature adipocytes induces the expression of a number of genes involved in the insulin signaling cascade, thereby improving insulin sensitivity. PPARγ is the master regulator of adipogenesis, thereby stimulating the production of small insulin-sensitive adipocytes(Leonardini et al., 2009). In a study with diabetic mice, decanoic acid (a fatty acid in MCT) acted as a partial agonist at the nuclear receptor

PPAR γ (Malapaka et al., 2012). Other agonists on PPAR γ are the antidiabetic drugs glitazones that are produced by the PPAR γ- Activation lead to improved insulin sensitivity and blood sugar control. The increased decanoic acid intake in KD (especially in MCT-based KD) could consequently be due to PPAR γ-activation have an effect similar to that of glitazones. The improvement in glycemic control appears to be associated with weight loss (Malapaka et al., 2012).

The result of the current study shows reduction in serum C Peptide (representing insulin) in prediabetes group, that's due to restriction of sugar which is mean stimulator for secretion of insulin (Campbell and Newgard, 2021)in the other hand, higher insulin secretion observed with oral glucose compared to intravenous infusion of glucose, which is due to incretin hormones. satiating effect of the KD affect the secretion incretins hormones released by entero-endocrine cells of gut. The reduction of secretion of incretins also reduce insulin secretion (Holst and Gromada, 2004).Production of the ketone bodies is regulated hormonally, with insulin having an inhibiting effect and glucagon having a stimulating effect. Suppression of insulin secretion by means of continuous carbohydrate restriction is therefore essential for ketone synthesis(Gulati et al., 2017)

The reduction of serum C Peptide and glucose will directly reduce the HOMA IR value which Demonstrate the insulin resistance and also increase the cell sensitivity to insulin as seen in increase of the value of HOMA S% that's calculated by HOMA 2 calculator(Research, 2020) . This evidence suggest that the KD can be used for treatment of prediabetes and also may use for treatment of diabetic patients (Mobbs et al.,2013, Westman et al., 2018).In particular, insulin, blood glucose levels and HOMA IR can be improved by KD (Shai et al., 2008).The efficacy on weight loss by KD as a therapy in obese

diabetic patients has also been successfully observed (Shai et al., 2008). Finally, KD might have positive effect on the mitochondria, since mitochondrial dysfunctions are also being discussed as possible causes of T2DM. (Mugabo et al., 2010)

5.3 Effect of KD on Lipid Profile

Result of this study showed a significant elevation of total serum cholesterol and TG in control groups, with no significant increase in the cholesterol level of the prediabetes group. These results can be explained by the mobilization of cholesterol and TG from adipose tissue to the liver for the synthesis of ketone bodies, which assets in rapid weight loss (Orio et al., 2008). Increased exogenous fat consumption may contribute to blood lipid elevation, Eating foods containing saturated and trans fats causes your body to produce even more LDL (Ma et al., 2021). The elevation of blood lipid profile is considered a major risk for cardiovascular diseases such as atherosclerosis, which may lead to coronary artery disease such as angina pectoris (Welty, 2013). Interestingly, the prediabetes patients benefited from KD since their LDL was lowered and their HDL was elevated significantly. This improvement in level of serum LDL and HDL might due to reduction of insulin and C-peptide level which decreased in the prediabetic group. The lower blood insulin level in prediabetic group could also be responsible in the decrease in level of LDL and increase of level of HDL, because insulin has stimulating effect on the key enzyme HMG-CoA reductase in cholesterol synthesis (Soares et al., 2021). Insulin increasing the rate of transcription of HMG-CoA enzyme, whereas glucagon acts by opposing this effect (Han, 2018). Furthermore, a direct inhibition of cholesterol production through the production of ketone bodies is conceivable, since both synthesis pathways run

via the connection HMG-CoA (Han, 2018). The serum LDL is associated with more severe risks of atherosclerosis (Pereira et al., 2019) ; a lower level of serum HDL is related may also to the elevation of blood TG and increases the chance of atherosclerotic plaque formation. Higher HDL levels are considered a novel biomarker for reducing risks of cardiovascular disease (Nicholls and Nelson, 2019).

5.4 Effect of Short Term KD on Liver and Kidney

No considerable changes were detected in the level ALP, AST, and ALT parameter opposite to findings of (Paoli et al., 2013b),who established that KD reduced liver enzyme levels but (Watanabe et al., 2020) showed increase in liver enzyme and confirmed the occurrence of steatosis and liver inflammation in mice model. A considerable increase of all Kidney function parameters (creatinine, uric acid, urea ,and BUN) was observed, which is the opposite with Kapelner and Vorsanger (2015), Bruci et al. (2020) who documented a reduction in the blood uric acid and no change in blood creatinine. The elevation of Kidney parameters is strongly related to kidney stones frequently noted in epileptic children performing KD (McNally et al., 2009, Bruci et al., 2020). furthermore, increasing animal protein consumption and excluding many fruit and vegetables will promote kidney stones (Tracy et al., 2014). The lower pH encourages kidney stone formation by increasing urinary calcium and reducing citrate (Crosby et al., 2021); it may increase the risk of albuminuria (Lin et al., 2010) and chronic kidney diseases (Kalantar-Zadeh and Fouque, 2018). Other observational studies confirmed similar findings (Lew et al., 2016, Mirmiran et al., 2020); they uncovered that high animal protein intake for an extended period would damage the glomerulus and lead to chronic kidney diseases (Haring et al., 2017).

5.5 Effect of KD on Oxidative Stress

The increased oxidative stress markers (MDA and NO) in the prediabetes group in this study were agree with the result of (Arsyad et al., 2020) but it was opposite to the result of (Rhyu and Cho, 2014). MDA is considered an oxidative stress biomarker synthesis from the peroxidation of polyunsaturated fats. The elevation of MDA levels could be triggered by the increase of reactive oxygen species (ROS). The ROS elevation could induce ROS damage to our cell membrane and DNA (Nazarewicz et al., 2007). ROS are also involved in myelin degradation, phagocytosis, damage of oligodendrocytes and axonal degeneration. Inflammation contributes to oxidative stress and conversely (Xu et al., 2022).The NO as well slightly increased in both prediabetes and control groups. This result was in line with findings of (Ma et al., 2018).

5.6 Effect of KD on level of IL10

The level of serum IL-10, an anti-inflammatory adipokine, was higher after applying KD in both control and prediabetes groups. The reason for the elevation of IL-10 may be due to the induction of gene expression of IL-10 by ketone bodies (Shaw et al., 2021). This elevation of IL-10 may be beneficial for humans since it is an anti-inflammatory cytokine. IL-10 can compensate for tissue damage by oxidative stress, which was enhanced by KD in this study increased. Since higher anti-inflammation factors are related with a lessening the risks of diabetic angiopathies, (Mugabo et al., 2010),Whereas (Burkitt, 2020) reveals that ketosis connected to increases in inflammatory cytokine TNF-α, which is a risk factor for vascular inflammation, and protein carbonyls. Likewise, the increase in ketone bodies is connected with an increase in lipid peroxidation, which is another risk factor for cardiovascular illnesses (Burkitt, 2020)

CONCLUSIONS

1- The KD is an effective method for controlling body weight.

2-The KD very useful for reducing insulin resistance in prediabetes individuals.

3-One of the most important issue is to inform and educate the individuals who performing KD since it is a restrictive diet and requires control to monitor the ketosis situation, which is key for the diet to exercise its main benefits.

4- The use of the KD is not always beneficial since it has been proven that it could be harmful in various Situation. It could cause damage kidney by causing kidney stone; It may cause chronic kidney diseases due to increase of kidney function parameters.

5-The KD also increases the oxidative stress and blood lipid profile which is considered a major risk for cardiovascular diseases. The disadvantage of KD outweighs their advantage.

5- The ketogenic diet increases the lipid profile of healthy obese individuals.

6- The Serum HDL increase and LDL decreased in prediabetes individuals.

RECOMMENDATIONS AND FUTURE WORKS

We recommend the followings:

1- Further studies are needed to investigate other adipokine such as gastrin, adiponectin, visfatin ,leptin, chermerin, resistin and nesfatin

2- Current data concerning the role of oxidative mechanisms in KD, the possibility of counteracting it by antioxidant administration plus an appropriate diet, and other parameters should be taken that related to oxidative stress, such as zinc, magnesium, selenium, glutathione reductase, ROS and SOD-1.

3- Additional research is required with a larger population, longer duration, and the inclusion of other disorders including T2DM and PCOS.

4- More investigation on different types of KD is needed to investigate the benefits and drawbacks of each type separately.

5- More research is needed to investigate the anti-inflammatory effect of KD by looking at other inflammatory biomarkers like IL2, IL6, TNFS, and CRP.

REFERENCES

ACHARYA, P., ACHARYA, C., THONGPRAYOON, C., HANSRIVIJIT, P., KANDURI, S. R., KOVVURU, K., MEDAURA, J., VAITLA, P., GARCIA ANTON, D. F. & MEKRAKSAKIT, P. 2021. Incidence and characteristics of kidney stones in patients on ketogenic diet: a systematic review and meta-analysis. *Diseases,* 9**,** 39.

AL-KHALIFA, A., MATHEW, T. C., AL-ZAID, N. S., MATHEW, E. & DASHTI, H. M. 2009. Therapeutic role of low-carbohydrate ketogenic diet in diabetes. *Nutrition,* 25**,** 1177-1185.

ALBRIGHT, A. L. & GREGG, E. W. 2013. Preventing type 2 diabetes in communities across the U.S.: the National Diabetes Prevention Program. *American Journal of Preventive Medicine,* 44**,** S346-51.

ALLAIN, C. C., POON, L. S., CHAN, C. S., RICHMOND, W. & FU, P. C. 1974. Enzymatic determination of total serum cholesterol. *Clinical chemistry* .475-470 ,20

ALTUVE, M. & SEVEREYN, E. 2019. Cluster Analysis Based on Fasting and Postprandial Plasma Glucose and Insulin Concentrations. *bioRxiv***,** 861468.

ARSYAD, A., IDRIS, I., RASYID, A. A., USMAN, R. A., FARADILLAH, K. R., LATIF, W. O. U., LUBIS, Z. I., AMINUDDIN, A., YUSTISIA, I. & DJABIR, Y. Y. 2020. Long-term ketogenic diet induces metabolic acidosis, anemia, and oxidative stress in healthy wistar rats. *Journal of nutrition metabolism* .2020

ATKINS, C. 2002. *Dr. Atkins' new diet revolution* ,USA, Harper.

BARHAM, D. & TRINDER, P. 1972. An improved colour reagent for the determination of blood glucose by the oxidase system. *Analyst,* 97**,** 142-145.

BATTELLI, M. G., BORTOLOTTI, M., POLITO, L. & BOLOGNESI, A. 2018. The role of xanthine oxidoreductase and uric acid in metabolic syndrome. *Biochimica et Biophysica Acta -Molecular Basis of Disease*.2565-2557 ,1864

BEKKOUCHE, L., BOUCHENAK, M., MALAISSE, W. & YAHIA, D. A. 2014. The Mediterranean diet adoption improves metabolic, oxidative, and inflammatory abnormalities in Algerian metabolic syndrome patients. *Hormone metabolic research,* 46**,** 274-282.

BERGMEYER, H., HERDER, M. & REF, R. 1986a. International federation of clinical chemistry (IFCC). *Journal of clinical chemistry and clinical biochemistry* .510-497 ,24.

BERGMEYER, H. U., HØRDER, M. & REJ, R. 1986b. International Federation of Clinical Chemistry (IFCC) Scientific Committee, Analytical Section: approved recommendation (1985) on IFCC methods for the measurement of catalytic concentration of enzymes. Part 2. IFCC method for aspartate aminotransferase (L-aspartate: 2-oxoglutarate aminotransferase, EC 2.6.1.1). *Journal of clinical chemistry and clinical biochemistry,* 24**,** 497-510.

BEST, T., FRANZ, D., GILBERT, D., NELSON, D. & EPSTEIN, M. 2000. Cardiac complications in pediatric patients on the ketogenic diet .*Neurology* .2330-2328 ,54

BLÜHER, M. 2019. Obesity: global epidemiology and pathogenesis. *Nature Reviews Endocrinology,* 15**,** 288-298.

BODENANT, M., MOREAU, C., SEJOURNE, C., AUVIN, S., DELVAL, A., CUISSET, J., DERAMBURE, P., DESTÉE, A. & DEFEBVRE, L. 2008 .Interest of the ketogenic diet in a refractory status epilepticus in adults. *Revue neurologique*.199-194 ,164.

BOLLA, A. M., CARETTO, A., LAURENZI, A., SCAVINI, M. & PIEMONTI, L. 2019. Low-carb and ketogenic diets in type 1 and type 2 diabetes. *Nutrients*.962 ,11 ,

BONDAR, R. J. & MEAD, D. C. 1974. Evaluation of glucose-6-phosphate dehydrogenase from Leuconostoc mesenteroides in the hexokinase method for determining glucose in serum. *Clinical chemistry*-586 ,20

.590

BOSTOCK, E., KIRKBY, K. C., TAYLOR, B. V & .HAWRELAK, J. A. 2020. Consumer reports of "keto flu" associated with the ketogenic diet. *Frontiers in nutrition***,** 20.

BOYLE, J. P., THOMPSON, T. J., GREGG, E. W., BARKER, L. E. & WILLIAMSON, D. F. 2010. Projection of the year 2050 burden of diabetes in the US adult population: dynamic modeling of incidence, mortality, and prediabetes prevalence. *Population Health Metrics,* 8**,** 29.

BREHM, B. J., SEELEY, R. J., DANIELS, S. R. & D'ALESSIO, D. A. 2003. A randomized trial comparing a very low carbohydrate diet and a calorie-restricted low fat diet on body weight and cardiovascular risk factors in healthy women. *The Journal of Clinical Endocrinology Metabolism*.1623-1617 ,88

BRUCI, A., TUCCINARDI, D., TOZZI, R., BALENA, A., SANTUCCI, S., FRONTANI, R., MARIANI ,S., BASCIANI, S., SPERA, G. & GNESSI, L. 2020. Very low-calorie ketogenic diet: a safe and effective tool for weight loss in patients with obesity and mild kidney failure. *Nutrients,* 12**,** 333.

BURKITT, M. J. 2020. An overlooked danger of ketogenic diets: Making the case that ketone bodies induce vascular damage by the same mechanisms as glucose. *Nutrition*110763 ,75

CAMPBELL, J. E. & NEWGARD, C. B. 2021. Mechanisms controlling pancreatic islet cell function in insulin secretion. *Nature reviews Molecular cell biology*, 22**,** 142-158.

CHARY, A. & HEDAYATI, M. 2022. Review of Laboratory Methods to Determine HDL and LDL Subclasses and Their Clinical Importance. *Reviews in Cardiovascular Medicine*, 23**,** 147.

CHOI, J. N., SONG, J. E., SHIN, J. I., KIM, H. D., KIM, M .J. & LEE, J. S.

2010. Renal stone associated with the ketogenic diet in a 5-year old girl with intractable epilepsy. *Yonsei medical journal,* 51**,** 457-459.

COHEN, R. M., SNIEDER, H., LINDSELL, C. J., BEYAN, H., HAWA, M. I., BLINKO, S., EDWARDS, R., SPECTOR, T. D. & LESLIE, R. D. G. 2006. Evidence for independent heritability of the glycation gap (glycosylation gap) fraction of HbA1c in nondiabetic twins. *Diabetes care,* 29**,** 1739-1743.

COOK, J. 1975. Factors Influencing the Assay of Creatinine: Prepared for the Association of Clinical Biochemists' Scientific and Technical Committee. *Annals of Clinical Biochemistry,* 12**,** 219-232.

CROSBY, L., DAVIS, B., JOSHI, S., JARDINE, M., PAUL, J., NEOLA, M. & BARNARD, N. D. 2021. Ketogenic Diets and Chronic Disease: Weighing the Benefits Against the Risks. *Frontiers in Nutrition,* 8.

DALBETH, N., CHOI, H. K., JOOSTEN, L. A., KHANNA, P. P., MATSUO, H., PEREZ-RUIZ, F. & STAMP, L. K. 2019. Gout (Primer). *Nature Reviews: Disease Primers*.5

DEARLOVE, D. J., FAULL, O. K., ROLLS ,E., CLARKE, K. & COX, P. J. 2019. Nutritional ketoacidosis during incremental exercise in healthy athletes. *Frontiers in physiology*290 ,10

DEEMER, S. E., PLAISANCE, E. P. & MARTINS, C. 2020. Impact of ketosis on appetite regulation—a review. *Nutrition Research*.11-1 ,77

DEFRONZO, R. A., FERRANNINI, E., GROOP, L., HENRY, R. R., HERMAN, W. H., HOLST, J. J., HU, F. B., KAHN, C. R., RAZ, I. & SHULMAN, G. I. 2015. Type 2 diabetes mellitus. *Nature reviews Disease primers,* 1**,** 1-22.

DELANAYE, P., CAVALIER, E & .POTTEL, H. 2017. Serum creatinine: not so simple! *Nephron*.308-302 ,136

ECHOUFFO-TCHEUGUI, J. B. & SELVIN, E. 2021. Prediabetes and what it

means: the epidemiological evidence. *Annual Review of Public Health,* 42**,** 59-77.

ELKINS, C., FRUH, S., JONES, L & .BYDALEK, K. 2019. Clinical practice recommendations for pediatric dyslipidemia. *Journal of Pediatric Health Care*.504-494 ,33

FABBRINI, E. & KLEIN, S. 2008. Fundamentals of cardipmetabolic risk factor reduction: achieving and maintaining weight loss with pharmacotherapy or bariatric surgery. *Clinical Cornerstone,* 9**,** 41-51.

FABINY, D. L. & ERTINGSHAUSEN, G. 1971. Automated reaction-rate method for determination of serum creatinine with the CentrifiChem. *Clinical chemistry,* 17**,** 696-700.

FÄNDRIKS, L. 2017 .Roles of the gut in the metabolic syndrome: an overview. *Journal of internal medicine*.336-319 ,281

FLANNICK, J., JOHANSSON, S. & NJØLSTAD, P. R. 2016. Common and rare forms of diabetes mellitus: towards a continuum of diabetes subtypes. *Nature Reviews Endocrinology,* 12**,** 394-406.

FLU, K. 2018. *The Truth About the Ketogenic Diet* [Online]. Available: https://movewellinhome.com/blog-posts/2018/6/3/the-ketogenic-diet-and-weight-loss [Accessed].

FORST, T., RAVE, K., PFUETZNER, A., BUCHHOLZ, R., POHLMANN, T ,.LÖBIG, M. & HEINEMANN, L. 2002. Effect of C-peptide on glucose metabolism in patients with type 1 diabetes. *Diabetes Care* ,25 .1097-1096

FOSSATI, P. & PRENCIPE, L. 1982. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clinical chemistry*.2080-2077 ,28

FRANKEL, H. M. & STAEHELI, J. C. 1992. Calculating body mass index. *Annals of Internal Medicine,* 117**,** 698-699.

FREEDMAN, D. S., HORLICK, M. & BERENSON, G. S. 2013. A comparison of the Slaughter skinfold-thickness equations and BMI in predicting body fatness and cardiovascular disease risk factor levels in children. *The American journal of clinical nutrition*.1424-1417 ,98

FRIEDEWALD, W. T., LEVY, R. I & .FREDRICKSON, D. S. 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical chemistry*, 18**,** 499-502.

FU, Z., GILBERT, E. R. & LIU, D. 2013. Regulation of insulin synthesis and secretion and pancreatic Beta-cell dysfunction in diabetes. *current diabetes reviews,* 9**,** 25-53.

FURUHASHI, M. 2020. New insights into purine metabolism in metabolic diseases: role of xanthine oxidoreductase activity. *American Journal of Physiology-Endocrinology Metabolism* ,319E827-E834.

GANO, L. B., PATEL, M. & RHO, J. M. 2014. Ketogenic diets, mitochondria, and neurological diseases. *Journal of lipid research* ,55 .2228-2211

GARROW, J. S. & WEBSTER, J. 1985. Quetelet's index (W/H2) as a measure of fatness. *International journal of obesity*.153-147 ,9

GAUTSCHI, M., WEISSTANNER, C., SLOTBOOM, J., NAVA, E., ZÜRCHER, T. & NUOFFER, J.-M. 2015. highly efficient ketone body treatment in multiple acyl-coa dehydrogenase deficiency–related leukodystrophy. *Pediatric Research,* 77**,** 91-98.

GERSHUNI, V. M., YAN, S. L. & MEDICI, V. 2018. Nutritional ketosis for weight management and reversal of metabolic syndrome. *Current nutrition reports*.106-97 ,7

GETAHUN, D., GETABALEW, M., ZEWDIE, D., ALEMNEH, T. & AKEBEREGN, D. 2 .019Urea metabolism and recycling in ruminants.

Biomedical Journal of Scientific & Technical Research, 20**,** 14790-14796.

GIUGLIANO, D., CERIELLO, A. & ESPOSITO, K. 2008. Glucose metabolism and hyperglycemia. *The American journal of clinical nutrition* 217 ,87S-222S.

GODAY, A., BELLIDO, D., SAJOUX, I., CRUJEIRAS, A. B., BURGUERA, B., GARCÍA-LUNA, P. P., OLEAGA, A., MORENO, B. & CASANUEVA, F. F. 2016. Short-term safety, tolerability and efficacy of a very low-calorie-ketogenic diet interventional weight loss program versus hypocaloric diet in patients with type 2 diabetes mellitus. *Nutrition diabetes* ,6e230-e230.

GULATI, S., MISRA, A., TIWARI, R., SHARMA, M., PANDEY, R. M. & YADAV, C. P. 2017. Effect of high-protein meal replacement on weight and cardiometabolic profile in overweight/obese Asian Indians in North India. *British journal of nutrition,* 117**,** 1531-1540.

GUTIÉRREZ, C., GALVÁN, A. & OROZCO, S. 2013. Ketogenic diets in the treatment of overweight and obesity. *Clinical Nutrition Hospital Dietetics*.111-98 ,33

GUZEL, O., UYSAL, U. & ARSLAN, N. 2019 .Efficacy and tolerability of olive oil-based ketogenic diet in children with drug-resistant epilepsy: A single center experience from Turkey. *european journal of paediatric neurology*.151-143 ,23

HAFIANE, A. & GENEST, J. 2015. High density lipoproteins :measurement techniques and potential biomarkers of cardiovascular risk. *BBA clinical*, 3**,** 175-188.

HALLBERG, S. J., MCKENZIE, A. L., WILLIAMS, P. T., BHANPURI, N. H., PETERS, A. L., CAMPBELL, W. W., HAZBUN, T. L., VOLK, B. M., MCCARTER, J. P. & PHINNEY, S .D. 2018. Effectiveness and

safety of a novel care model for the management of type 2 diabetes at 1 year: an open-label, non-randomized, controlled study. *Diabetes Therapy,* 9**,** 583-612.

HAN, K. H. 2018. Functional implications of HMG-CoA reductase inhibition on glucose metabolism. *Korean Circulation Journal*.963-951 ,48

HAN, X., ZHANG, S., CHEN, Z., ADHIKARI, B. K., ZHANG, Y., ZHANG, J., SUN, J. & WANG, Y. 2020. Cardiac biomarkers of heart failure in chronic kidney disease. *Clinica Chimica Acta,* 510**,** 298.310-

HARING, B., SELVIN, E., LIANG, M., CORESH, J., GRAMS, M. E., PETRUSKI-IVLEVA, N., STEFFEN, L. M. & REBHOLZ, C. M. 2017. Dietary protein sources and risk for incident chronic kidney disease: results from the Atherosclerosis Risk in Communities (ARIC) Study. *Journal of Renal Nutrition*.242-233 ,27

HOLMAN, R., HINES, G., KENNEDY, I., STEVENS, R., MATTHEWS, D. & LEVY, J. 2004. A calculator for HOMA. *Diabetologia* ,47A222.

HOLST, J. J. & GROMADA, J. 2004. Role of incretin hormones in the regulation of insulin secretion in diabetic and nondiabetic humans. *American Journal of Physiology-Endocrinology Metabolism* ,287E199-E206.

IKEGAMI, H., HIROMINE, Y. & NOSO, S. 2022. Insulin-dependent diabetes mellitus in older adults: Current status and future prospects . *Geriatrics Gerontology International*.553-549 ,22

JAFFÉ, M. 1886. Ueber den Niederschlag, welchen Pikrinsäure in normalem Harn erzeugt und über eine neue Reaction des Kreatinins. *Walter de Gruyter,* 10 391-400.

JONES, A. & HATTERSLEY, A. 2013. The clinical utility of C-peptide measurement in the care of patients with diabetes. *Diabetic medicine* ,30 .817-803

KALANTAR-ZADEH, K. & FOUQUE, D. 2018. Nutritional management of chronic kidney disease. *The New England journal of medicine,* 378**,** 584-585.

KAPELNER, A & .VORSANGER, M. 2015. Starvation of cancer via induced ketogenesis and severe hypoglycemia. *Medical hypotheses,* 84**,** 162-168.

KNOWLER, W. C., BARRETT-CONNOR, E., FOWLER, S. E., HAMMAN, R. F., JOHN M LACHIN, WALKER, E. A. & NATHAN, D. M. 2002. Reduction in the Incidence of Type 2 Diabetes with Lifestyle Intervention or Metformin. *The New England Journal of Medicine,* 346**,** 393-403.

KOPIN, L. & LOWENSTEIN, C. J. 2017. Dyslipidemia. *Annals of internal medicine,* 167**,** ITC81-ITC96.

KOSSOFF, E. H. & DORWARD, J. L. 2008. The modified Atkins diet. *Epilepsia,* 49**,** 37-41.

KOSSOFF, E. H., ZUPEC-KANIA, B. A., AMARK, P. E., BALLABAN-GIL, K. R., CHRISTINA BERGQVIST, A., BLACKFORD, R., BUCHHALTER, J. R., CARABALLO, R. H., HELEN CROSS, J. & DAHLIN, M. G. 2009. Optimal clinical management of children receiving the ketogenic diet: recommendations of the International Ketogenic Diet Study Group. *Epilepsia*.317-304 ,50

KUSHNER, R. F. 2005. Low-Carbohydrate Diets, Con: The Mythical Phoenix or Credible Science? *Nutrition in clinical practice*.16-13 ,20

KWITEROVICH JR, P. O., VINING, E. P., PYZIK, P., SKOLASKY JR, R. & FREEMAN, J. M. 2003. Effect of a high-fat ketogenic diet on plasma levels of lipids, lipoproteins, and apolipoproteins in children. *Jama* ,290 .920-912

LECHNER, K., MCKENZIE, A. L., KRÄNKEL, N., VON SCHACKY, C.,

WORM, N., NIXDORFF, U., LECHNER, B., SCHERR, J., WEINGÄRTNER, O. & KRAUSS, R. M. 2020. High-risk atherosclerosis and metabolic phenotype: the roles of ectopic adiposity, atherogenic dyslipidemia, and inflammation. *Metabolic syndrome related disorders.*.185-176 ,18

LEONARDINI, A., LAVIOLA, L., PERRINI, S., NATALICCHIO, A. & GIORGINO, F. 2009. Cross-Talk between PPARgamma and Insulin Signaling and Modulation of Insulin Sensitivity. *PPAR Res,* 2009**,** 818945.

LEW, Q., JAFAR, T., KOH, H ,.JIN, A., CHOW, K., YUAN, J. & KOH, W. 2016. Red meat intake and risk of ESRD *Journal of the American Society of Nephrology*.312-304 ,28

LIN, J., HU, F. B. & CURHAN, G. C. 2010. Associations of diet with albuminuria and kidney function decline. *Clinical Journal of the American Society of Nephrology*.843-836 ,5

LONOWSKI, S. & LEVINS, P. 2020. A unique case of prurigo pigmentosa related to ketogenic diet. *Dermatology Online Journal*.26

LORENZO, C., OKOLOISE, M., WILLIAMS, K., STERN, M. P. & HAFFNER, S. M. 2003. The metabolic syndrome as predictor of type 2 diabetes: the San Antonio heart study. *Diabetes care,* 26**,** 3153-3159.

LOVRIĆ, J., MESIĆ, M., MACAN, M., KOPRIVANAC, M., KELAVA, M. & BRADAMANTE, V. 2008. Measurement of malondialdehyde (MDA) level in rat plasma after simvastatin treatment using two different analytical methods. *Hrcak Portal of scientific journals of Croatia,* 110**,** 63 - 68.

MA, D., WANG, A. C., PARIKH, I., GREEN, S. J., HOFFMAN, J. D., CHLIPALA, G., MURPHY, M. P., SOKOLA, B. S., BAUER, B. & HARTZ, A. 2018. Ketogenic diet enhances neurovascular function with

altered gut microbiome in young healthy mice. *Scientific reports*.10-1 ,8

MA, Y., ZHA, J., YANG, X., LI, Q., ZHANG, Q., YIN, A., BEHARRY, Z., HUANG, H., HUANG, J. & BARTLETT, M. 2021. Long-chain fatty acyl-CoA synthetase 1 promotes prostate cancer progression by elevation of lipogenesis and fatty acid beta-oxidation. *Oncogene*.1820-1806 ,40

MADDALONI, E., BOLLI, G. B., FRIER, B. M., LITTLE, R. R., LESLIE, R. D., POZZILLI, P. & BUZZETTI, R. 2022. C-peptide determination in the diagnosis of type of diabetes and its management: A clinical perspective. *Diabetes, Obesity Metabolism*.1926-1912 ,24

MALAPAKA, R. R., KHOO, S., ZHANG, J., CHOI, J. H., ZHOU, X. E., XU, Y., GONG, Y., LI, J., YONG, E.-L. & CHALMERS, M. J. 2012. Identification and mechanism of 10-carbon fatty acid as modulating ligand of peroxisome proliferator-activated receptors. *Journal of Biological Chemistry*.195-183 ,287

MAYANS, L. 2015. Metabolic syndrome: insulin resistance and prediabetes. *FP essentials*.16-11 ,435

MCGOWAN, M. W., ARTISS, J. D., STRANDBERGH, D. R. & ZAK, B. 1983. A peroxidase-coupled method for the colorimetric determination of serum triglycerides. *Clinical chemistry*.542-538 ,29

MCINTYRE, H. D., CATALANO, P., ZHANG, C., DESOYE, G., MATHIESEN, E. R. & DAMM, P. 2019. Gestational diabetes mellitus. *Nature reviews Disease primers*.19-1 ,5

MCNALLY, M. A., PYZIK, P. L., RUBENSTEIN, J. E., HAMDY, R. F. & KOSSOFF, E. H. 2009. Empiric use of potassium citrate reduces kidney-stone incidence with the ketogenic diet. *Pediatrics* ,124e300-e304.

MILLER, W. G., MYERS, G. L., SAKURABAYASHI, I., BACHMANN, L. M., CAUDILL, S. P., DZIEKONSKI, A., EDWARDS, S., KIMBERLY, M. M., KORZUN, W. J., LEARY, E. T., NAKAJIMA, K.,

NAKAMURA, M., NILSSON, G., SHAMBUREK, R .D., VETROVEC, G. W., WARNICK, G. R. & REMALEY, A. T. 2010. Seven direct methods for measuring HDL and LDL cholesterol compared with ultracentrifugation reference measurement procedures. *Clinical Chemistry,* 56**,** 977-86.

MIRMIRAN, P., YUZBASHIAN, E., AGHAYAN, M., MAHDAVI, M., ASGHARI, G. & AZIZI, F. 2020. A prospective study of dietary meat intake and risk of incident chronic kidney disease. *Journal of Renal Nutrition*.118-111 ,30

MOSS, D., HENDERSON, A. & KACHMAR, J. 1987. *Enzymes in: Tietz NW, ed. Fundamentals of clinical chemistry,* Philadelphia: WB Saunders.

MUGABO, Y., LI, L. & RENIER, G. 2010. The connection between C-reactive protein (CRP) and diabetic vasculopathy. Focus on preclinical findings. *Current diabetes reviews*.34-27 ,6

MURRAY, R. & KAPLAN, A. 1984 .*Clinical Chemistry: Theory, Analysis, Correlation,* Mosby/Elsevier.

MYETTE-CÔTÉ, É., DURRER, C., NEUDORF, H., BAMMERT, T. D., BOTEZELLI, J. D., JOHNSON, J. D., DESOUZA, C. A. & LITTLE, J. P. 2018. The effect of a short-term low-carbohydrate, high-fat diet with or without postmeal walks on glycemic control and inflammation in type 2 diabetes: a randomized trial. *American Journal of Physiology-Regulatory, Integrative Comparative Physiology,* 315**,** R1210-R1219.

NAZAREWICZ, R. R., ZIOLKOWSKI, W., VACCARO, P. S & . GHAFOURIFAR, P. 2007. Effect of short-term ketogenic diet on redox status of human blood. *Rejuvenation Research*.440-435 ,10

NEELEY, W. E. 1972. Simple automated determination of serum or plasma glucose by a hexokinase/glucose-6-phosphate dehydrogenase method. *Clinical Chemistry*.515-509 ,18

NELSON, R. H. 2013. Hyperlipidemia as a risk factor for cardiovascular disease. *Primary Care,* 40**,** 195-211.

NICHOLLS, S. J. & NELSON, A. J. 2019. HDL and cardiovascular disease. *Pathology*.147-142 ,51

NORRIS, J. M., JOHNSON, R. K. & STENE, L. C. 2020. Type 1 diabetes—Early life origins and changing epidemiology. *The lancet Diabetes endocrinology*.238-226 ,8

NYMO, S., COUTINHO, S., JØRGENSEN, J., REHFELD, J. F., TRUBY, H., KULSENG, B. & MARTINS, C. 2017. Timeline of changes in appetite during weight loss with a ketogenic diet. *International Journal of Obesity*.1231-1224 ,41

O'NEILL, B. & RAGGI, P. 2020. The ketogenic diet: Pros and cons. *Atherosclerosis,* 292**,** 119-126.

OLIVEIRA, C. L., MATTINGLY, S., SCHIRRMACHER, R., SAWYER, M. B., FINE, E. J. & PRADO, C. M. 2018. A nutritional perspective of ketogenic diet in cancer: a narrative review. *Journal of the Academy of Nutrition Dietetics*.688-668 ,118

ORIO, F., GIALLAURIA, F., PALOMBA, S., MANGUSO, F., ORIO, M., TAFURI, D ,.LOMBARDI, G., CARMINA, E., COLAO, A. & VIGORITO, C. 2008. Metabolic and cardiopulmonary effects of detraining after a structured exercise training programme in young PCOS women. *Clinical endocrinology*.981-976 ,68

PACKARD, C. J., BOREN, J. & TASKINEN, M-.R. 2020. Causes and consequences of hypertriglyceridemia. *Frontiers in endocrinology* ,11 .252

PAOLI, A. 2014. Ketogenic diet for obesity: friend or foe? *International journal of environmental research public health*.2107-2092 ,11

PAOLI, A., RUBINI, A., VOLEK, J. & GRIMALDI, K. 2013a. Beyond

weight loss: a review of the therapeutic uses of very-low-carbohydrate (ketogenic) diets. *European journal of clinical nutrition*.796-789 ,67

PAOLI, A., RUBINI, A., VOLEK, J. S. & GRIMALDI, K. A. 2013b. Beyond weight loss: a review of the therapeutic uses of very-low-carbohydrate (ketogenic) diets. *European Journal of Clinical Nutrition,* 67**,** 789-796.

PARTSALAKI, I., KARVELA, A. & SPILIOTIS, B. E. 2012. Metabolic impact of a ketogenic diet compared to a hypocaloric diet in obese children and adolescents. *ournal of Pediatric Endocrinology Metabolism*.704-697 ,25

PASARICA, M. & DHURANDHAR, N. V. 2019. Pharmacological Management of the Patient with Obesity. *Lifestyle Medicine.* CRC Press.

PASCUAL, J. M., LIU, P., MAO, D., KELLY, D. I., HERNANDEZ, A., SHENG, M., GOOD, L. B., MA, Q., MARIN-VALENCIA, I. & ZHANG, X. 2014. Triheptanoin for glucose transporter type I deficiency (G1D): modulation of human ictogenesis, cerebral metabolic rate, and cognitive indices by a food supplement. *JAMA neurology*.1265-1255 ,71

PEREIRA, L., NASCIMENTO, J., RÊGO, J., CANUTO, K., CRESPO-LOPEZ, M., ALVAREZ-LEITE, J., BAYSAN, A. & ORIÁ, R. 2019. Apolipoprotein E, periodontal disease and the risk for atherosclerosis: a review. *Archives of Oral Biology*.212-204 ,98

PÉREZ-GUISADO, J. 2010. Los triglicéridos de cadena media, agentes para perder peso, inducir la cetosis y mejorar la salud en general. *International Journal of Obesity Latest Journal,* 8**,** 124-9.

POFF, A. M., KOUTNIK, A. P. & EGAN, B. 2020. Nutritional ketosis with ketogenic diets or exogenous ketones: features, convergence, and divergence. *Current Sports Medicine Reports*.259-251 ,19

PRADEEP, G., VIKRAM, B. & SHARMA, D. 2019. Biomarkers to Assess Liver Function in Various Types of Liver Diseases. *International*

Journal of Clinical Biomedical Research.31-28

PUNDIR, C., JAKHAR, S. & NARWAL, V. 2019. Determination of urea with special emphasis on biosensors: A review. *Biosensors Bioelectronics*.50-36 ,123

RADER, D. J. & HOVINGH, G. K. 2014. HDL and cardiovascular disease. *The Lancet*.625-618 ,384

RAMIREZ-CAMACHO, A., MEAVILLA, S., CATALAN, N., GUTIERREZ, A. & CAMPISTOL, J. 2011. Experience with ketogenic diet as treatment for refractory epilepsy. *Revista de Neurologia*-524 ,53 .530

REAVEN, G. M. 2005. The insulin resistance syndrome: definition and dietary approaches to treatment. *Annual Review of Nutrition*-391 ,25 ,. .406

REID, C. A., MULLEN, S., KIM, T. H. & PETROU, S. 2014. Epilepsy, energy deficiency and new therapeutic approaches including diet. *Pharmacology therapeutics*.201-192 ,144

RESEARCH, O. 2020. *The HOMA2 Calculator* [Online]. Available: https://www.optimaldx.com/blog/homa2-calculator [Accessed November 8, 2020].

RHYU, H.-S. & CHO, S.-Y. 2014. The effect of weight loss by ketogenic diet on the body composition, performance-related physical fitness factors and cytokines of Taekwondo athletes. *Journal of exercise rehabilitation* .326 ,10

RIBEIRO, C. B., RAMOS, F. M., MANTHEY, J. A. & CESAR, T. B. 2019. Effectiveness of Eriomin® in managing hyperglycemia and reversal of prediabetes condition: A double-blind, randomized, controlled study. *Phytotherapy Research*.1933-1921 ,33

ROCHLANI, Y., POTHINENI, N. V., KOVELAMUDI, S. & MEHTA, J. L.

2017. Metabolic syndrome: pathophysiology, management, and modulation by natural compounds. *Therapeutic Advances in Cardiovascular Disease,* 11**,** 215-225.

ROEHL, K., FALCO-WALTER, J., OUYANG, B. & BALABANOV, A. 2019. Modified ketogenic diets in adults with refractory epilepsy: Efficacious improvements in seizure frequency, seizure severity, and quality of life. *Epilepsy Behavior*.118-113 ,93

SALGADO, A. L., CARVALHO, L., OLIVEIRA, A. C., SANTOS, V. N., VIEIRA, J. G. & PARISE, E. R. 2010. Insulin resistance index (HOMA-IR) in the differentiation of patients with non-alcoholic fatty liver disease and healthy individuals. *Arquivos de Gastroenterologia*, 47**,** 165-9.

SAMPSON, E. J., BAIRD, M. A., BURTIS, C., SMITH, E. M., WITTE, D. & BAYSE, D. D. 1980. A coupled-enzyme equilibrium method for measuring urea in serum: optimization and evaluation of the AACC study group on urea candidate reference method. *Clinical chemistry* ,26 .826-816

SANKARANENI, R. M. & SINGH, S. 2022. Ketogenic Diet and Pediatric Epilepsy–A Historical Review (1920–1964)(P11-7.001). *Neurology*, 98

SASLOW, L. R., MASON, A. E., KIM, S., GOLDMAN, V., PLOUTZ-SNYDER, R., BAYANDORIAN, H., DAUBENMIER, J., HECHT, F. M. & MOSKOWITZ, J. T. 2017. An online intervention comparing a very low-carbohydrate ketogenic diet and lifestyle recommendations versus a plate method diet in overweight individuals with type 2 diabetes: a randomized controlled trial. *Journal of medical Internet research* ,19e5806.

SÁYAGO-AYERDI, S., VAQUERO, M., SCHULTZ-MOREIRA, A., BASTIDA, S. & SÁNCHEZ-MUNIZ, F. 2008. Utilidad y controversias del consumo de ácidos grasos de cadena media sobre el metabolismo

lipoproteico y obesidad. *Nutrición Hospitalaria,* 23**,** 191-202.

SCHADE, D. S., SHEY, L. & EATON, R. P. 2020. Cholesterol review: a metabolically important molecule. *Endocrine Practice*.1523-1514 ,26

SHAI, I., SCHWARZFUCHS, D., HENKIN, Y., SHAHAR, D. R., WITKOW, S., GREENBERG, I., GOLAN, R., FRASER, D., BOLOTIN, A. & VARDI, H. 2008. Weight loss with a low-carbohydrate, Mediterranean, or low-fat diet. *New England Journal of Medicine,* 359**,** 229-241.

SHARMA, U., PAL, D. & PRASAD, R. 2014. Alkaline phosphatase: an overview .*Indian Journal of Clinical Biochemistry*.278-269 ,29

SHAW, D. M., MERIEN, F., BRAAKHUIS, A., KEANEY, L. & DULSON, D. K. 2021. Adaptation to a ketogenic diet modulates adaptive and mucosal immune markers in trained male endurance athletes. *Scandinavian Journal of Medicine Science in Sports*.152-140 ,31

SOARES, F. L. P., RAMOS, M. H., GRAMELISCH, M., DE PAULA PEGO SILVA, R., DA SILVA BATISTA, J., CATTAFESTA, M. & SALAROLI, L. B. 2021. Intuitive eating is associated with glycemic control in type 2 diabetes .*Eating Weight Disorders-Studies on Anorexia, Bulimia Obesity*.608-599 ,26

SPIETH, L. E., HARNISH, J. D., LENDERS, C. M., RAEZER, L. B., PEREIRA, M. A., HANGEN, S. J. & LUDWIG, D. S. 2000. A low–glycemic index diet in the treatment of pediatric obesity .*Archives of Pediatrics*

Adolescent Medicine.951-947 ,154

STAFSTROM, C. E. & RHO, J. M. 2012. The ketogenic diet as a treatment paradigm for diverse neurological disorders. *Frontiers in pharmacology* .59 ,3

STEVEN, S. & TAYLOR, R. 2015. Restoring normoglycaemia by use of a

very low calorie diet in long-and short-duration Type 2 diabetes. *Diabetic Medicine*.1155-1149 ,32

STOCKER, R. K., AUBRY, E. R., BALLY, L., NUOFFER, J.-M. & STANGA, Z. 2019a. Ketogene Diät: evidenzbasierte therapeutische Anwendung bei endokrinologischen Erkrankungen. *Praxis*.

STOCKER, R. K., BALLY, L., NUOFFER, J. & STANGA, Z. 2019b. Ketogenic diet and its evidence-based therapeutic implementation in endocrine diseases. *Praxis,* 108**,** 541-553.

STONE, S. J. 2022. Mechanisms of intestinal triacylglycerol synthesis. *Biochimica et Biophysica Acta -Molecular Cell Biology of Lipids*.159151

SUDHAKARAN, S., YAZDANI, L., WHEELAN, K. R. & RAO, P. K. The ketogenic diet and the QT interval. Baylor University Medical Center Proceedings, 2020. *Taylor & Francis*, 77-79.

SUGIUCHI, H., IRIE, T., UJI, Y., UENO, T., CHAEN, T., UEKAMA, K. & OKABE, H. 1998. Homogeneous assay for measuring low-density lipoprotein cholesterol in serum with triblock copolymer and α-cyclodextrin sulfate. *Clinical chemistry*.531-522 ,44

SUMITHRAN, P. & PROIETTO, J. 2008. Ketogenic diets for weight loss: a review of their principles, safety and efficacy. *Obesity Research Clinical Practice*.13-1 ,2

SUN, J., ZHANG, X., BRODERICK, M. & FEIN, H. 2003. Measurement of Nitric Oxide Production in Biological Systems by Using Griess Reaction Assay. *Sensors,* 3**,** 276-284.

TADA, H., NOHARA, A., INAZU, A., SAKUMA, N., MABUCHI, H. & KAWASHIRI, M.-A. 2018. Sitosterolemia, hypercholesterolemia, and coronary artery disease. *Journal of Atherosclerosis Thrombosis* RV17024.

TAMAOKU, K., UENO, K., AKIURA, K. & OHKURA, Y. 1982. New

water-soluble hydrogen donors for the enzymatic photometric determination of hydrogen peroxide. II. N-ethyl-N-(2-hydroxy-3-sulfopropyl) aniline derivatives. *Chemical Pharmaceutical Bulletin* ,30 .2497-2492

TIETZ, N. 1983. IFCC method for the measurement of catalytic concentration of enzymes Part 5. IFCC method for alkaline phosphatase. *clinical chemical biochemistry* .748-731 ,21

TIETZ, N. W. 1995. Clinical guide to laboratory tests. *Clinical guide to laboratory tests*.

TIFFANY, T., JANSEN, J., BURTIS, C., OVERTON, J. & SCOTT, C. 1972. Enzymatic kinetic rate and end-point analyses of substrate, by use of a GeMSAEC fast analyzer. *Clinical Chemistry*.840-829 ,18

TILG, H. & MOSCHEN, A. R. 2008. Inflammatory mechanisms in the regulation of insulin resistance. *Molecular medicine,* 14**,** 222-231.

TIYYAGURA, S. R. & SMITH, D. A. 2006. Standard lipid profile. *Clinics in laboratory medicine*.732-707 ,26

TRACY, C. R., BEST, S., BAGRODIA, A., POINDEXTER, J. R ,.ADAMS-HUET, B., SAKHAEE, K., MAALOUF, N., PAK, C. Y. & PEARLE, M. S. 2014. Animal protein and the risk of kidney stones: a comparative metabolic study of animal protein sources. *The Journal of urology,* 192**,** 137-141.

URBAIN, P. & BERTZ, H. 2016. Monitoring for compliance with a ketogenic diet: what is the best time of day to test for urinary ketosis? *Nutrition metabolism*.6-1 ,13

VACCAREZZA, M., AGUSTINHO, A., ALBERTI, M. J., ARGUMEDO, L., ARMENO, M., BLANCO, V., BOUQUET, C., CABRERA, A., CARABALLO, R & .CARAMUTA, L. 2016. National consensus on the modified Atkins diet. *Revista de Neurologia*.376-371 ,62

VENUGOPAL, S. K., MOWERY, M. L. & JIALAL, I. 2022. *C peptide*, StatPearls

VILLALÓN RODRÍGUEZ, D. 2020. *Advantages and disadvantages of the ketogenic diet* .MSc, University of Seville.

VON AH MORANO, A. E., DORNELES, G. P., PERES, A. & LIRA, F. S. 2020. The role of glucose homeostasis on immune function in response to exercise: The impact of low or higher energetic conditions. *Journal of cellular physiology*.3188-3169 ,235

VORIN, V., MOUROT, J., WEILL, P., ROBIN, G., PEINIAU, P. & MOUNIER, A. 2003. Effet de l'apport d'acides gras oméga 3 dans l'alimentation du porc sur les performances de croissance et la qualité de la viande. *Journées Rech. Porcine*.256-251 ,35

WARNICK, G. R., NAUCK, M. & RIFAI, N. 2001. Evolution of methods for measurement of HDL-cholesterol: from ultracentrifugation to homogeneous assays. *Clinical chemistry*.1596-1579 ,47

WATANABE, M., TOZZI, R., RISI, R., TUCCINARDI, D., MARIANI, S., BASCIANI, S., SPERA, G., LUBRANO, C. & GNESSI, L. 2020. Beneficial effects of the ketogenic diet on nonalcoholic fatty liver disease: A comprehensive review of the literature. *Obesity Reviews* ,21 e13024.

WELTY, F. K. 2013. How do elevated triglycerides and low HDL-cholesterol affect inflammation and atherothrombosis? *Current cardiology reports* .13-1 ,15

WHELESS, J. W. 2008. History of the ketogenic diet. *Epilepsia*, 49**,** 3-5.

WILCOX, G. 2005. Insulin and insulin resistance. *Clinical biochemist reviews*.19 ,26

WIRTH, A., WABITSCH, M. & HAUNER, H. 2014. The prevention and treatment of obesity. *Deutsches Arzteblatt international*, 111**,** 705-713.

WOHLFAHRT-VEJE, C., TINGGAARD, J., WINTHER, K., MOURITSEN, A., HAGEN, C., MIERITZ, M., DE RENZY-MARTIN, K., BOAS, M., PETERSEN, J. & MAIN, K. 2014. Body fat throughout childhood in 2647 healthy Danish children: agreement of BMI, waist circumference, skinfolds with dual X-ray absorptiometry. *European journal of clinical nutrition*.670-664 ,68

XU, T., LIU, C., DENG, S., GAN ,L., ZHANG, Z., YANG, G.-Y., TIAN, H. & TANG, Y. 2022. The roles of microglia and astrocytes in myelin phagocytosis in the central nervous system. *Journal of Cerebral Blood Flow Metabolism* 0271678X221137762.

YAMAGISHI, K. & ISO, H. 2017. The criteria for metabolic syndrome and the national health screening and education system in Japan. *Epidemiology health* .39

YANCY JR, W. S., VERNON, M. C. & WESTMAN, E. C. 2003. A pilot trial of a low-carbohydrate, ketogenic diet in patients with type 2 diabetes. *Metabolic syndrome related disorders,* 1**,** 239-243.

YOSTEN, G. L. & KOLAR, G. R. 2015. The physiology of proinsulin C-peptide: unanswered questions and a proposed model. *Physiology* ,30 .332-327

ZHANG, Y., LUO, S., GONG, Z., FENG, X., WANG, Z., ZHU, H. & WANG, Y. 2 .018Improvement of hyperandrogenism, oligo-ovulation, and ovarian morphology in a patient with polycystic ovary syndrome: possible role of ovarian wedge resection. *Gynecological Endocrinology* .463-460 ,34

پوختە

ئەم تـوێژینەوەیە ئەنجـام دراوە بـۆ زانینـی کـاریگەری ڕیجێمـی کیتـۆ بـۆ سـەر هەندێ لە پشـکینینەکانی خـوێن کە تـایبەتە بە ڕێـژەی شـەکرەی خـوێن و فرمـانی جەرگ و گـورچیلە و مـاددەی ئۆکسـێنەر دژەهەوکـردن لە کەسـانی قەڵەوی تەندروسـت و کەسـانی پـێش شـەکرە. ئەم تـوێژینەوەیە لە یەکـی لە چێشـتخانە تایبەتمەنـدەکانی کیتـۆ لە شـاری هەولێـر-کوردسـتان ئەنجـام لە نێـوان. مـانگی 9 سـاڵی 2021 بـۆ مـانگی 10 سـاڵی 2022، لە دوای ئەنجامـدانی جیاکـاری بـو دانـان و دوورخسـتنەوەی کەسـەکان، تەنهـا 58 کەس بەردەوامبـوون لە تـوێژینەوەکە کە پۆڵین کرابوون بۆ دوو گرووپ
گرووپـی یەکەم: پێـک هـاتبوو لە 34 کەسـی قەڵەوی تـووش نەبـوو بە شـەکرە، گرووپـی دووەم: 24 کەسـی بەرکەوتە بـۆ تـووش بـوو بە شـەکرە. خـوێن لە هەمـوو بەشـداربوان وەرگیـرا پـێش دەسـت پـی کردنـی ڕیجیمـی کیتـۆ و 3 مانـگ دوای ڕیجێمـی کیتـۆ. پاشـان ڕێـژەی شـەکرە و پشـکنینەکانی فرمـانی جـگەر و گـورچیلە و مـاددەی ئۆکسـێنەر وئینتەرلیکـونی 10 پێـوراوە لە پـیش کێتـۆ و دوای 3 مـانگی کیتـۆ. پـاش بەراوردکردنـی پشـکینەکان لە پـێش کیتـۆ و دوای کیتـۆ لە هەردوو گـرووپ. تێکـڕای جیـاوازی پیشـکینەکانی هەردوو گـرووپەش بەراورد کـرا بەیەک. دەرکەوت کە ڕیجیمـی کیتـۆ بژاردەیەکـی باشـە بـۆ کـۆنترۆڵ کردنـی کێشـی لەش هەروەهـا دەرکەوت کە ڕێجیمـی کیتـۆنی کـارگەری ئەرێنـی هەیە لەسـەر کەمکـردنەوەی بەرگـری ئەنسـۆلین لەو کەسـانەی کە بە دەسـت پـێش شـەکرەی خـوێن دەنـاڵنن. لەگەل ئەوەشـدا ڕێجیمـی کیتـۆنی زیانیشـی هەیە وەکـو ئەوەی ئەنجامەکـان نیشـانی دەدەن.چـونکە دەبێـتە هـۆی بەرزبـونەوەی چەوری خـوێن کە هۆکـاری سـەرەکی نەخۆشـیەکانی دڵە.لە هەمـان کاتـدا فەرمانەکـانی گـورچیلەش بەرزدەکـاتەوە کە ئەمـانش هوکـاری نەخوشـیە درێژخایاناکـانی گـورچیلەن هەروەهـا هۆکـاری دروسـتبونی بەردی گورچیلەو نەخوشیەکانی دڵن.

کاریگەری ڕێجیمی کیتۆ لەسەر بەرگری ئەنسۆلین و ماددەی ئۆکسێنەر و ئینتەرلیکونی 10 و هەندێک پارامێتری بایۆکیمیایی تر لە کەسانی قەڵەوی پێش شەکرە

نامەیەکە

پێشکەش بە ئەنجومەنی کۆلێژی تەکنیکی تەندروستی و پزیشکی هەولێر کراوە لە

زانکۆی پۆلیتەکنیکی - هەولێر

وەک بەشێک لە پێداویستیەکانی بەدەستهێنانی پلەی ماستەر لە بەشی شیکاری نەخۆشیەکان

لە لایەن

احمد عبد الرزاق باپیر

بەکالۆریۆس لە زیندەوەرزانی - زانکۆی سەلاحەدین- هەولێر-2013

بە سەرپەرشتیاری

پ.ی.د. گوران قادر عثمان

د. برهان احمد صالح

هەولێر – کوردستان

حوزەیران-2022