العلاقة بين انزيم الكرياتين كاينيز ومضادات الاكسدة في مرضى السكري نوع I ونوع II

رسالة تقدم بها
إلى مجلس كلية العلوم – جامعة بابل
و هي جزء من متطلبات نيل درجة الماجستير في
علوم الكيمياء الحياتية

محمود حسين هدوان
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٢٠٠٢

THE CORRELATION BETWEEN CREATINE KINASE ACTIVITY AND ANTIOXIDANTS IN DIABETES MELLITUS TYPE I AND II

A Thesis

Submitted to the College of Science
University of Babylon
In Partial Fulfillment of the Requirements
For the Degree of Master of Science in
Biochemistry (Clinical Biochemistry)

By

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بسم الله الرحمن الرحيم

((اللهُ لا اله ألا هو الحيّ القَيّوم لا تأخذه سِنةٌ ولا نَوْمٌ لَهُ مَا في السموات وَ ما في الأرض مَنْ ذا الذي يشْفَعُ عِنْدَهُ إلا بإذنه يَعْلَمُ ما بينَ أيديهم وما خلْفَهُم ولا يحيطُونَ بشيء مِنْ عِلمِهِ إلا بِما شآءَ وِسِع كُرسيّهُ السمواتِ والأرض ولا يؤده حِفْظُهُما وهُو العليُّ العظيمُ))

صدق الله العلي العظيم

البقرة /٢٥٥

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In The Name of God Most Gracious Most Merciful

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CERTIFICATION

I certify that this thesis was prepared under my supervision at the Department of chemistry, College of science in Babylon University, in partial requirements for the Degree of Master of Science in Biochemistry and this work has never been published anywhere.

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الخلاصة

جرى قياس فعالية انزيم الكرياتين كاينيز في امصال دم اشخاص اصحاء ظاهرياً (ذكور واناث) باستخدام طريقة طيفية. ثم قيست فعالية انزيم الكرياتين كاينيز في امصال دم اشخاص مصابين بمرض السكري نوع I (ذكور، اناث) ونوع II (ذكور، اناث)، لتحديد دور هذا الانزيم بصفته مؤشراً كيميائياً حياتياً لمرضى السكري.

جرى كذلك فصل متناظرات انزيم الكرياتين كاينيز باستخدام كروموتوغرافيا التبادل الايوني من عمود الفصل المتكون من DEAE- Cellulose ثم حسبت مستويات هذه المتناظرات باستخدام طريقة طيفية.

تم قياس مستويات بعض مضادات الاكسدة مثل الكلوتاثيون وحامض اليوريك كما تم قياس اشكال الدهون في المصل بالاضافة الى MDA كنتيجة الى اكسدة الدهون الحاصلة بفعل الاجهاد التاكسدي المرتبط بمرض السكري.

مقارنة بمجموعة الاصحاء، وجد بان فعالية انزيم الكرياتين كاينيز تتناقص في مرضى السكري نوع I ونوع II. يختلف تأثير السكري على مستويات المتناظرات الأنزيمية لمرضى الكرياتين كاينيز (النوع العضلي MM-CK والقلبي MB-CK يتناقصان، بينما النوع الدماغي BB-CK لا يتأثر نسبياً).

وجد بان مستويات حامض اليوريك والكلوتاثيون تتناقص في أمصال مرضى السكري نوع I ونوع II مقارنة بقيمتها في أمصال الأصحاء. ربما يعود الانخفاض في مستوى مضادات الأكسدة الى حقيقة مقاومتها للإجهاد التاكسدي الحاصل بفعل مرضى السكري.

لدى مرضى السكري نوع I ونوع II يقل مستويات البروتين الدهني عالي الكثافة في أمصال دم مرضى السكري مقارنة بمستوياتها في أمصال دم الأشخاص

الأصحاء. كما وجد بان مرضى السكري يزيد مستويات البروتين الدهني واطئ الكثافة وكذلك البروتين الدهني ذو الكثافة الواطئة جداً. كما ان هناك زيادة معنوية في مستوى الكوليسترول الكلي وكذلك الكليسرايد الثلاثي.

وجد أيضا زيادة معنوية في مستوى المالونداي الديهايد الناتج بفعل أكسدة الدهون، وهذا قد يدل على زيادة الاجهاد التاكسدي الحاصل بفعل زيادة فصائل الأوكسجين الفعالة وكذلك فصائل النتروجين الفعالة.

ABSTRACT

The activity of creatine Kinase (CK) estimated, in sera of healthy controls (٣١ males, ١٩ females), in sera of patients with diabetes mellitus type I(٢١ male,٩ female) and type II (١٥male,٩ female). Then, CK isoenzyme MM- CK, MB- CK and BB-CK separated by using a column of DEAE- Cellulose. Spectrophotometric method has been used to evaluate the levels of this isoenzyme.

The concentrations of antioxidant variables such as glutathione, uric acid and lipid profile with addition to MDA were also assayed to measure the oxidative stress associated with Diabetes.

Compared with healthy controls, CK activity was found to be significantly decreasing in patients with Diabetes Mellitus; CK isoenzyme levels fluctuated between decrease and relatively constant in patients with Diabetes mellitus ((Diabetes mellitus decrease MM-CK and MB-CK activity)).

Glutathione, uric acid was found to be reduced when compared with those of healthy controls. The depletion in antioxidant concentrations may be due to their protective role against oxidative stress.

It was found that, Diabetes has lower HDL-c levels when compared with healthy controls, but higher in LDL-c, vLDL-c, TC, and TG. MDA, also increases in diabetes mellitus as a result of lipid peroxidation; that may be belong to the increased oxidative stress as a result of the increase in reactive oxygen species (ROS) and/ or reactive nitrogen species (RNS).

CK activity could be considered a biochemical marker for Diabetes mellitus.

Contents

(٢.٣١٪)

Chapter One

(١-١)General Introduction

Diabetes mellitus can be defined as a group of metabolic disorders of carbohydrate metabolism due to glucose under utilized, producing hyperglycemia [١]. There are three types of diabetes mellitus, type I and type II, Type I (Juvenile or insulin-dependent diabetes mellitus) is characterized by the autuimmune destruction of the pancreatic beta Cells [٣]. Type II diabetes (non-insulin dependent diabetes mellitus) which represents over ٨٠٪ of clinical cases [٢] Generally developed at later time from patient, the case of type II is insulin resistance [٤]. The reasons behind this resistance to insulin involve environmental causes and genetic causes [٥]. The diagnosis of diabetes mellitus depends only on proof of hyperglycemia (Table ١-١). The diagnosis of type I is easy because hyperglycemia appears severe and accompanied with serious metabolic disorders. In type II the diagnosis is difficult because glucose levels in serum may be mild; its importance to identify this type because the development of the complications accompanying it [٦].The third type called Gestational Diabetes Mellitus (GDM).GDM is carbohydrate intolerance of variable severity with onset or first recognition during the present pregnancy (i.e., diabetic women who become pregnant are not induced in this category).The occurrence of GDM is between ١٪ and ٥٪ (٣٪ is the most-often-quoted figure) In the United State, about ٩٠٠٠٠ women with GDM give birth each year, and as many as ٥٠٪may remained unidentified. About ٣٠٪ of women with GDM develop diabetes mellitus within ٢٠ years after delivery, but carbohydrate tolerance may revert to and stay normal after delivery [٧].GDM is also associated with a higher incidence of complication in the pregnancy (e.g., toxemia) , a higher risk of recurrence of

diabetes with following pregnancies , and an increased risk of diabetes in the mother ٥ to ١٠ years after parturition [٧].

Table (١.١) Criteria for the diagnosis of diabetes mellitus (with apology from [٥,٧])

Diabetes mellitus in non pregnant adults Any one of the following is diagnostic:-	
١-	Classical symptoms of diabetes and unequivocal elevation of plasma glucose
٢-	Elevated fasting glucose on more than one occasion venous plasma ≥١٤٠ mg/dl
٣-	Sustained elevated glucose concentration during the OGTT on move than one occasion venous plasma ≥ ٢٠٠ mg/dl
Impaired Glucose tolerance in non pregnant adults three criteria must be met:-	
١-	Fasting plasma glucose ≥ ١٤٠ mg/dl
٢-	½-hr,١-hr or(١.٥-hr OGTT plasma glucose ≥ ٢٠٠ mg/dl
٣-	٢-hr OGTT plasma glucose concentrations between ١٤٠and ٢٠٠ mg/dl
Diabetes mellitus in children Either ١ or ٢ is considered diagnostic of diabetes:-	
١-	Classical symptoms and a random plasma glucose > ٢٠٠ mg/dl
٢- A B -	In asymptomatic individuals:- A fasting glucose value of ≥١٤٠ mg/dl and Sustained elevated glucose concentration (≥ ٢٠٠ mg/dl) during the OGTT on more than one occasion.
Impaired glucose tolerance in children Two criteria should be met:-	
١-	Fasting plasma glucose concentration of < ١٤٠ mg/dl

٢-	Plasma glucose value ٢ hr after receiving oral glucose of > ١٤٠ mg/dl

(١-٢) Free Radicals in Medicine: -

It is difficult nowadays to open a medical journal without seeing some papers on the role of free radicals in humane diseases. These species concern with over ١٠٠-conditions [١٢]. Reactive Oxygen Species (ROS) are the most famous of this species which due to oxidative stress. Sies described the oxidative stresses as a change in pro-oxidant antioxidant balance in favor of the pro-oxidant state [١٣] see Fig (١ –١).

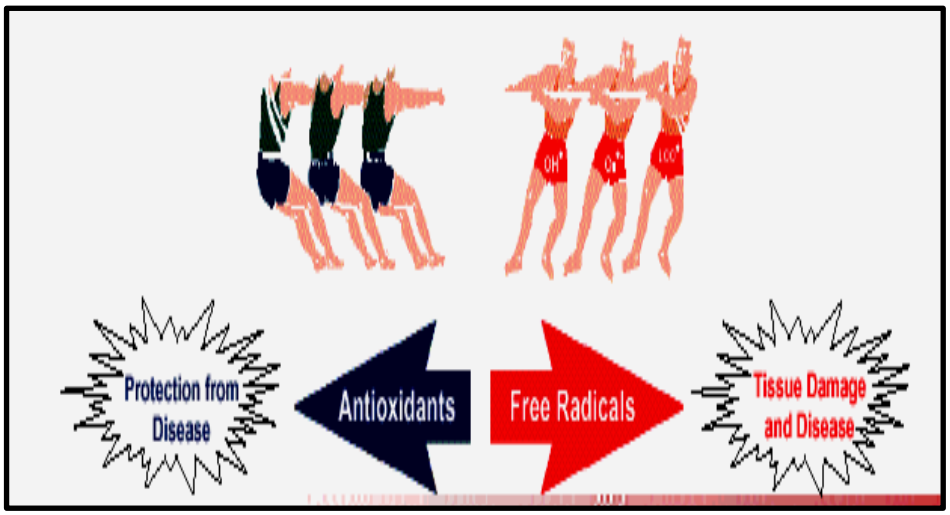


Fig (١-١) The relation between free radicals and antioxidants [١٥]

Antioxidant is defined as any substance that, when presented at low concentration compared with an oxidizable substrate, prevents or inhibits oxidation of the oxidizable substrate [١٣].

Free radicals are not always harmful. They also serve useful substances in the human body [١٣]. Several researches indicate that the oxygen radicals in living system are very necessary species in the maturation process of cellular structures. In addition, white blood cells destroy invading pathogenic microbes by the release of free radicals as part of body's defense mechanism against disease. Thus, the complete elimination of these radicals would not only be unnecessary, but also harmful [١٤].

(١-٣) The General Reactions of Free Radicals

A free radical is simply defined as any atom or molecule capable of independent existence that contains one or more unpaired electron [١٤]. Examples of free radicals are methyl radical ($\dot{C}H_3$, carbon centered radical), Nitric Oxide N=O$^{\cdot}$ in which the unpaired electron is delocalized between atoms, and thiyl radical ($R\dot{S}$, a sulphur centered radical).

Radical can react with other molecules or atom in a number of ways: -

١- Addition

$$\dot{X} + Y \rightarrow [X - Y]^{\bullet}$$

٢-Electron donation

$$\dot{X} + Y \rightarrow \dot{Y} + \overset{+}{X}$$

٣-Electron removal

$$X^{\bullet} + Y \rightarrow X^{-} + Y^{+}$$

٤-When two radicals meet a chain reaction can be terminated

$$X^{\bullet} + X^{\bullet} \rightarrow X_2$$

$$X^{\bullet} + Y^{\bullet} \rightarrow X - Y$$

The term reactive oxygen species is included also with non-radical derivatives of oxygen such as hydrochlorous acid (HOCl) and hydrogen peroxide (H_2O_2) [١٤].

(١-٤) Free Radical Production

There are more than one mechanism to produce free radicals;It is produced in living organism by two mechanisms; reaction of transition metals ion with hydrogen peroxide and heterolytic fission of water molecule by background exposure to ionizing radiation [١٦]. Hydroxyl radical is avery reactive species that attacks all biological molecules, usually setting off the chain of free radical reaction.

Supper oxide radical ($O_2^{-\bullet}$) is much less reactive than O˙H, but has a high sensitivity for a number of biological targets. Thus ($O_2^{-\bullet}$) reacts with nitric oxide to give peroxynitrite .[١٧] Nitric oxide and / or its derivative causes the relaxation of a smooth muscle cell in vessel wall. Thus ($O_2^{-\bullet}$) can act as a vasoconstrictor.

The considerable debate in the literature is the interaction between ($O_2^{-\bullet}$) and NO• due to its damaging effect on cells [١٨]. For example, peroxynitrite decomposes to form ˙OH in reaction independent of transition metal ion [١٧]. ($O_2^{-\bullet}$) When converted to protonated form ($HO_2^{\bullet}$) will be more reactive *in vitro* but has not yet been shown to mediate damaging effect *in vivo* [١٩].

(١-٥) Antioxidant System- :

Antioxidants are substances (molecule or atom) found in food or in the body at low concentration. An antioxidant has more than one function to prevent the oxidative sequence. They may act by preventing the generation of radical or inhibiting any free radicals that are generated [٢٠,٢١]. Tissue's defense against free

radical include primary antioxidant (catalase, superoxide dismutase, glutathion peroxidase) secondary antioxidant (glutathion, albumin, uric acid… etc) and essential radical scavengers such as vitamin C (in aqueous phase) and vitamin E (in the lipo-soluble phase)[٢١].

Enzymatic Defense: -

١- Super oxide dismutase (SOD)(EC ١.١٥.١.١)
This enzyme acts on the spontaneous dismutation of the super oxide ion to hydrogen peroxide [٢٢].

$$2\overset{-\bullet}{O}_2 + 2\overset{+}{H} \xrightarrow{SOD} H_2O_2 + O_2$$

There are three isoenzyme of SOD (An enzyme contains copper and zinc in the cytoplasm and manganese in mitochondria).

٢- catalase (EC(١.١١.١.٦)
This enzyme dissociates hydrogen peroxide as follows [٢٣]: -

$$H_2O_2 + H_2O_2 \xrightarrow{Cat} O_2 + 2H_2O$$

٣-Glutathione and glutathione enzymes (GSH-PX EC ١.١١.١.٩)

For the survival of animals, must be a high concentration from reduced glutathion (GSH) and low levels from oxidized form (GSSG) .[٢٣]

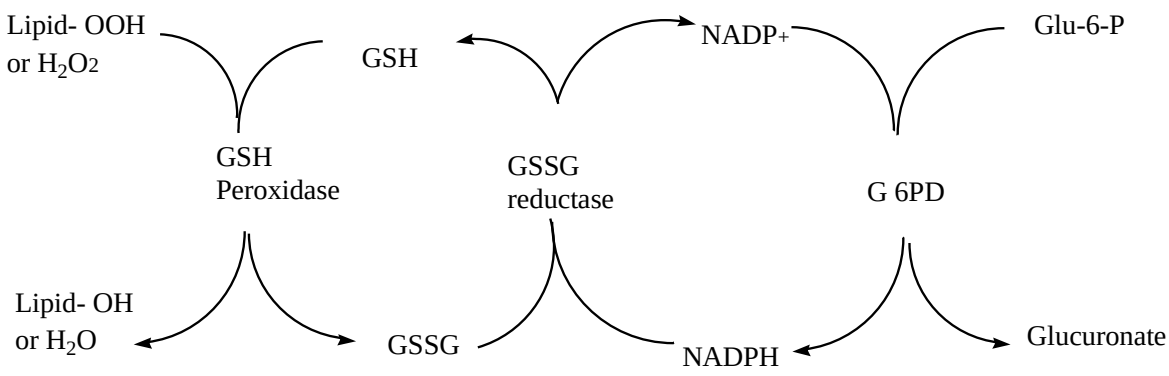
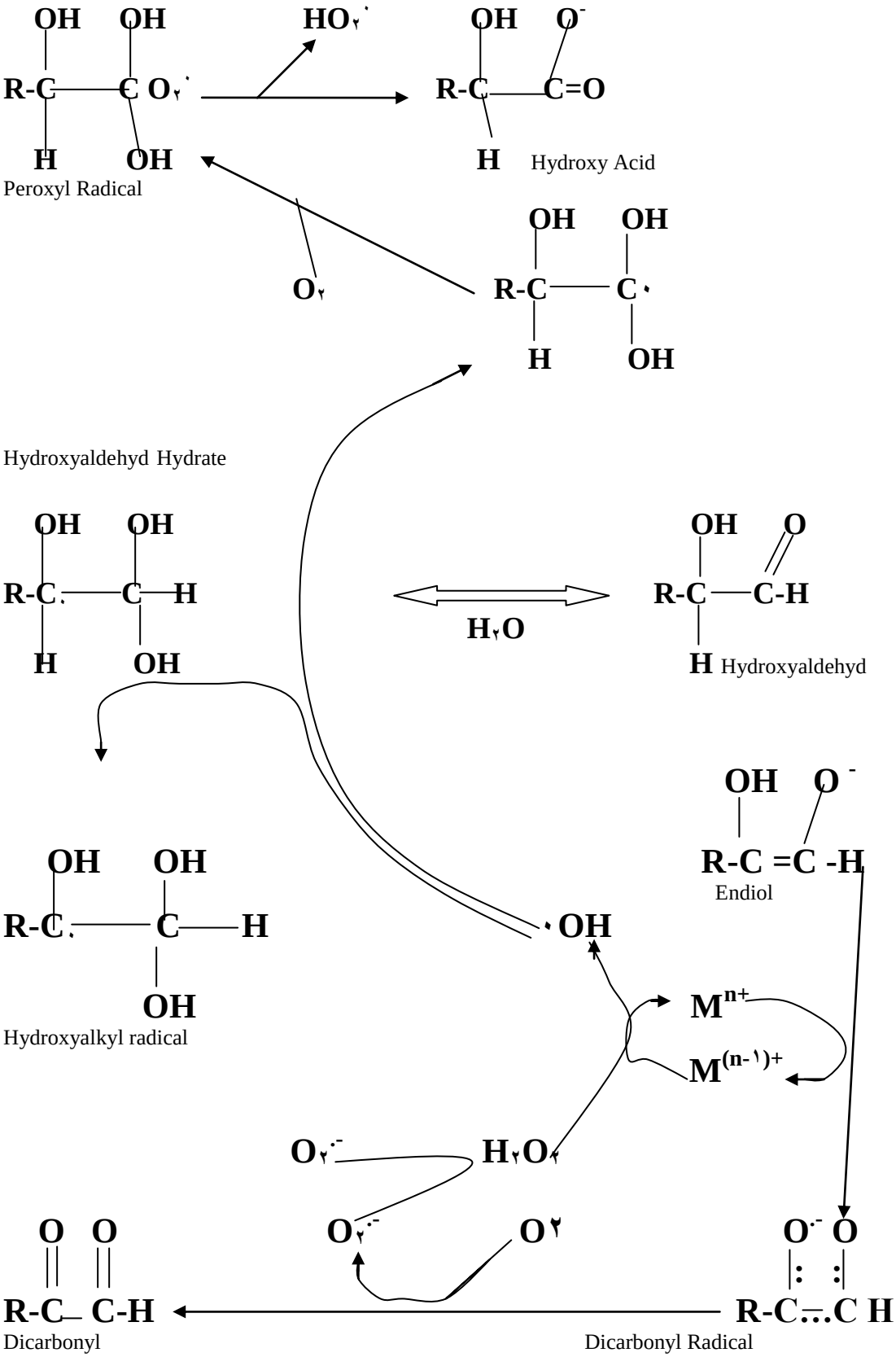


Fig (١-٢)Glutathione and glutathione enzymes (with apology from [٢٣])

(١-٧) Hyperglycemia and Free Radicals

Although the diabetes control and complications trial describe hyperglycemia as a risk factor for the development of diabetes complication [٢٤]. There is no consensus about the pathogenic link between hyperglycemia and complication [٢٥]. There are number of hypotheses on the origin of complications, including advanced glycation end product (AGE) hypothesis [٢٦,٢٧], oxidative stress [٢٨,٢٩,٣٠], reductive stress [٣١], carbonyl stress [٣٢] and increased protein kinase activity [٣٣]. These hypotheses overlap and interact with others, AGE formation and altered polyol pathway activity may be due to oxidative stress, which may be due to the acceleration of AGE formation [٢٥].

In this work, an attempt has been made to understand the relation between oxidative stress and diabetes complications. Hyperglycemia can increase the levels of free radicals through different pathways such as protein glycation, autoxidation glycation, protein kinase and increase in the polyol pathway. Glucose enolizes by the autoxidation process. This process entails the reduction of oxygen, to produce oxidizing intermediates, such as $O_2^{-\cdot}$, $O\cdot H$, H_2O_2 and α-keto aldehydes [٣٤]. See fig (١ –٣)[٣٦].



Fig(١-٣) The process of autoxidation of monosaccharide, showing how free radicals are produced when excess glucose is present [٣٦] .

These molecules can damage important biomolecules such as protein and lipids. The oxidizing intermediates formed by autoxidation process is proposed to be the case of some of the structures found in diabetes. This reaction is often catalyzed by transition metals and even with it, the reaction is very slow. These keto aldehyde products glyccation attach to proteins in a process called labile glycation[٣٥]. Glucose may also undergo glycation directly, where a schiff base is formed from acovalently bond between glucose and protein. Schiff's base undergoes rearengement to form an amadori adduct. Ammadori adducts can then decompose to produce deoxyglucosones, which are considerably more reactive than the sugar they are derived from it (fig ١-٤) .

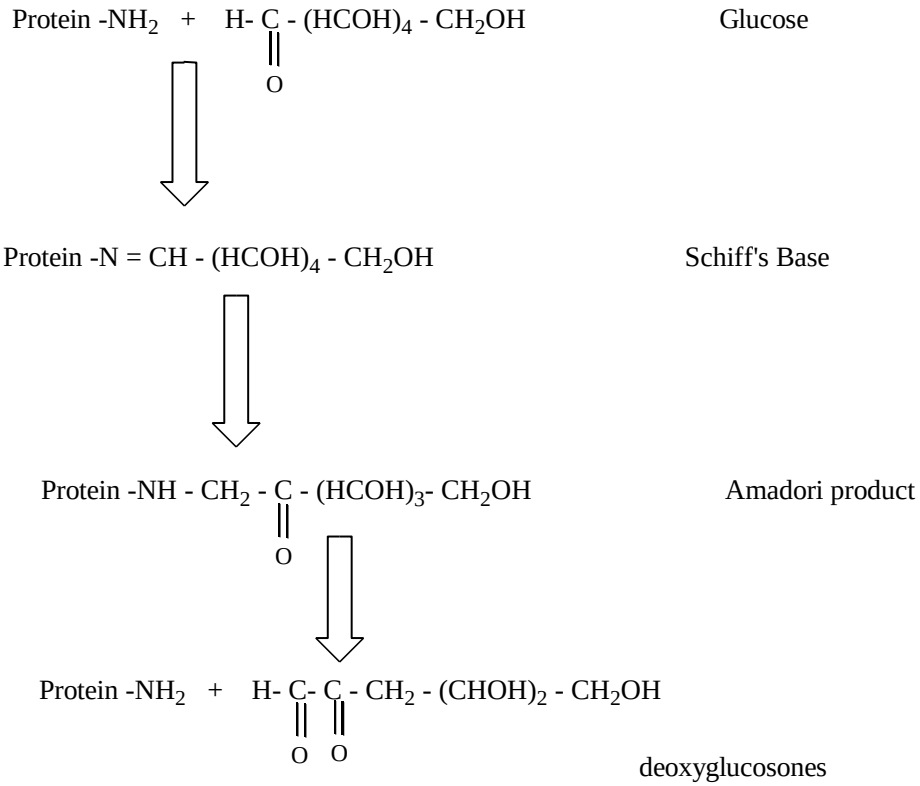
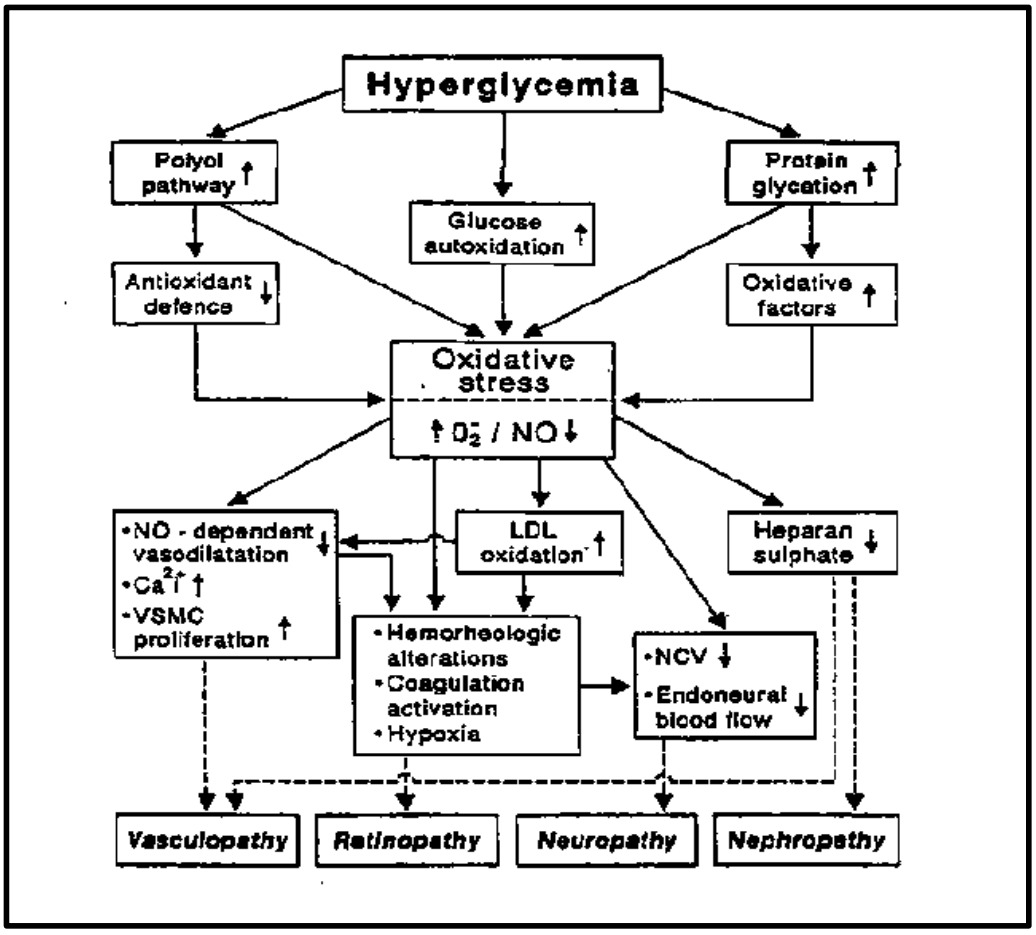


Fig (١-٤) Glycation process and subsequent degradation of glycation products (with apology from [٣٦])

These reactive ketoaldehyde may react with other protiens to form advanced glycation endproducts (AGE) or maillard products[٣٦]. An increase in the concentration of glucose contributes as an indicator to increase the activity of two enzymes used in the polyol pathway, aldose reductase and sorbitol dehyderogenase.The increasing activity of these two enzymes causes the increase of the concentration of both sorbitol and fructose. This increase activity is also due to the decrease of the ratio NADPH: $NADP^+$ and can cause changes throughout various systems(pentose phosphate pathway) in the cell such as NADH: NAD^+ . The increase in the NADH: NAD^+ ratio called hyperglycemic pseudohypoxia, may cause an increase in free radicals production which may due be to ischemia. It may also reduce glycolysis which results in reduced pyruvate levels[٣٨]. The decrease of the amount of NADPH may cause inhibition to enzymes which are NADPH – dependent and lead to the storage of the NADPH available for the many pathways that concerned with it.

(١-٧) Diabetes Complication and Free Radicals

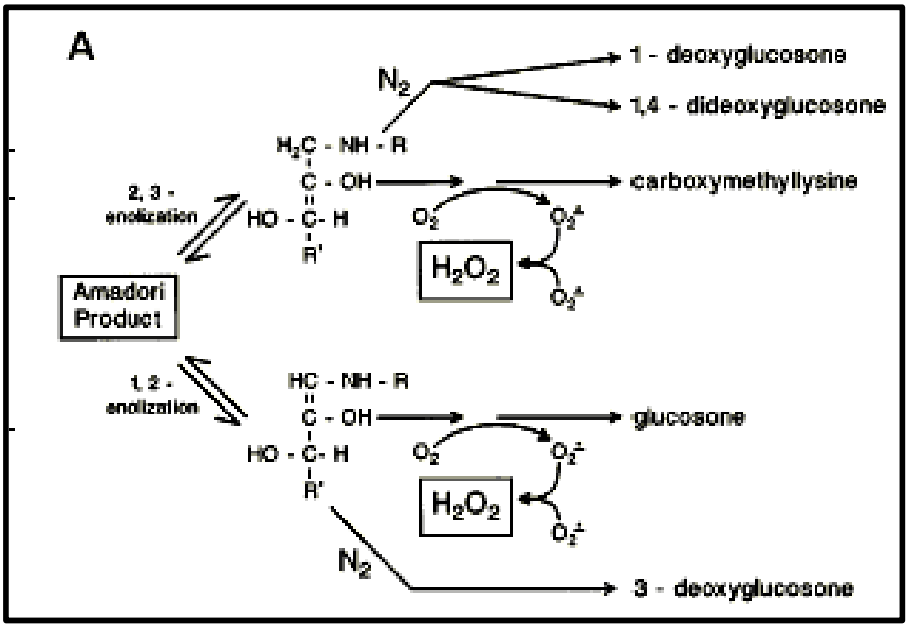
Many evidences have indicated that some biochemical pathway forcibly associated with hyperglycemia (non-enzymatic glycosylation, glucose autoxidation,polyol pathway)which may be due to increased free radicals production [٣٩] (fig ١–٥) .



Fig(١-٥) Possible links between hyperglycemia induced oxidative stress and diabetic complications. Solid lines indicate experimentally established links. A genetic predisposition through reduced antioxidant status may be present (dashed lines). NCV nerve conduction velocity; VSMC vascular smooth muscle cell. ((with apology from [٣٩]))

(١-٧-١)Free Radicals Generated by Non Enzymatic Glycosyation

Amadori products ensues exposure to non-enzymatic glycosylation of protein .These compounds implied in the formation of H_2O_2 *in vitro*[٤٠].In two pathways, amadori products could form H_2O_2 .Fig (١–٦).



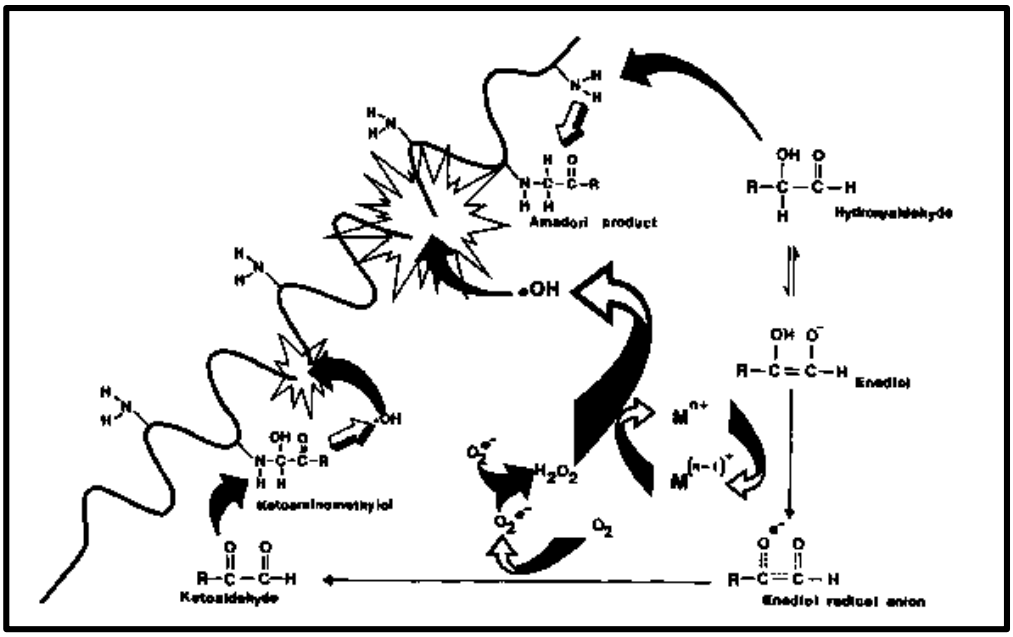
Fig(١-٦) Degradation of Amodari Product and H_2O_2 Formation. (H_2O_2 Formation can be Generated via both ١.٢ and ٢.٣ Enolization and Oxidation of the Enolate Anion). (with apology from [٤٠])

The first pathway is ١,٢-enolization pathway, which leads to ٣-deoxyglacosone formation under anaerobic conditions. When there are suitable electron acceptor, enolization would occur to form H_2O_2 and glucosone. The second pathway is ٢,٣ enolization pathway, which leads to ١-deoxyglucosone and putative ١,٤ deoxyglucosone[٤٠]. Under oxidative conditions, The ٢,٣ enediol is though to produce H_2O_2 and carboxy methyllysine. A major and highly reactive intermediate in the non-enzymatic glycosylation is ٣-deoxyglucosone and has a potent cross-linker responsible for the polymerization of protein to AGEs[٤٠].

AGEs tend to stay as linkage on long-lined macromolecules in tissues. The abnormalities of all tissues function produce cross-linking AGE – protein with other macromolecules in tissues[٤١].

(١-٧-٢) Free Radicals Generated by Autoxidation:-

In addition to direct glycosylation reaction, monosaccharide and fructose lysine can spontaneously reduce molecular oxygen under stable physiological conditions[٤٢]. Superoxide, hydroxyl radical and hydrogen peroxide produce as a results to reduced oxygen in the autoxidation reaction. All this species can damage lipids and proteins, through cross-linking and fragmentation .See fig(١-٧).



Fig(١-٧) The contribution of glucose autoxidation and hydroxyl radical production to glucose induced damage. The contribution of ketoaldehyde- amine Autoxidation to site-specific damage is suggested. (with apology from [٣٦])

Free radicals also accelerate the formation of advanced glycosylation end products, which in turn is due to generating more free radicals. This process is

called as glucose autoxidation[٤٣]. Although the importance of this process *in vivo* is only indirect, but there is some evidence *in vivo* that transition –metal chelating agents can prevent the oxidation process in animal diabetes[٤٤].

(١-٧-٣) Free Radicals Generated by Polyol Pathway:-

Polyol pathway can also produce free radicals ,which occurs because elevated glucose levels and due to increment intra cellular sorbitol and fructose content ,that's, induce aldose reductase and sorbitol dehydrogenase activity fig(١-٨).

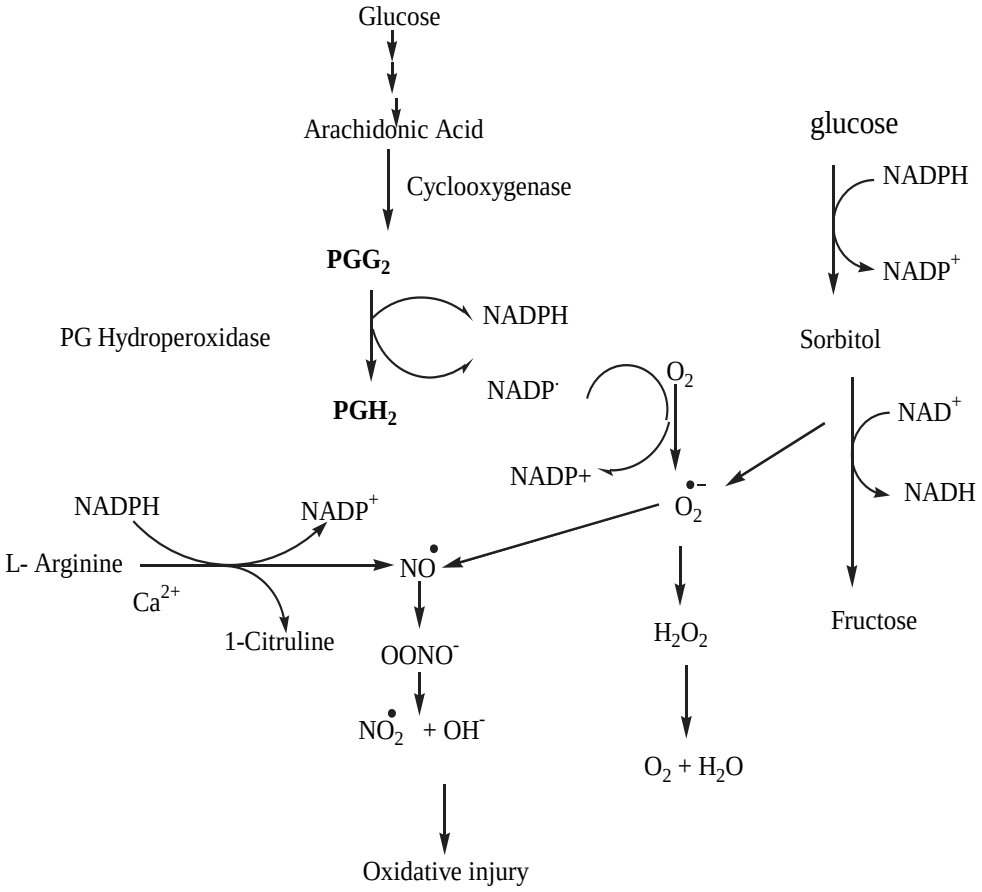


Fig (١-٨) The Polyol Pathway: aldose reductase catalyzes the reduction of glucose by NADPH to sorbitol which can, in turn, be oxidated to fructose by sorbitol dehydrogenase (SDH) leading to redox imbalance (NAD^+ / NADH ratio). An increase in NAD^+/ NADH ratio is linked to O_2 formation via the reduction of PGG٢ to prostoglandin H_2 (PGH_2) by prosto glandine hydroperoxidase that use NADH or NADPH as a reducing substrate. (with apology from [٤٣]).

Oxidation of sorbitol to fructose is coupled with reduction of NADPH . $\dot{O}_2^-$ formation occurs via the reduction of prostaglandin G٢ (PGG٢) to prostaglandin H٢ by prostaglandin hydroperoxidase that use NADH or NADPH as a reducing cosubstrate, that is linked with an increase NAD+/NADH ratio[٤٣].

(١-٨) Free Radical in the Pathogenesis of IDDM:-

IDDM appears as a result to the autoimmune beta cell destruction in pancreatic islets[٤٥].At normal conditions, beta cells secrete insulin in response to the increase of serum glucose. The decrease and finally total loss of insulin secretion is linked to destruction of β cell. Increasing evidence suggests that free radicals play as important factors in the β cell destruction and loss insulin.These evidences include:-

(١) It is true to say that ((hydrogen peroxide, nitric, nitric oxide and super oxide are toxic to the human and rat isletes *in vitro*))[٤٥].

(٢) The two most commonly used drugs in developing the diabetic animal models are aloxan and streptozotocin.These compounds is used because their ability to selectively damage the insulin- secreting beta cells of glucose-inducing insulin secretion. Streptozotocin releases nitric oxide in isolated pancreatic islets[٤٦], Aloxan stimulates superoxide production in beta cells[٤٧].

(٣) Vitamin E decreased the nitric oxide toxicity in the pancreatic islets *in vitro*[٤٨]. All these evidences refer to the potential role of free radicals in the β-cell destruction.

β-cells are prone to be destroyed by free radicals, that is related to the low antioxident enzyme nature[٤٨]. Macrophages, T cells, natural killer cells and other immune-effect cell are believed to produce free radicals that cause damage to B-cells[٤٩]. Free radicals have two mechanism in Beta – cell destruction.

(١) Infiltration of macrophages produce superoxide as primary free radicals, which can convert to more active radical such as hydroxyl radical which attacks cellular membrane and causes DNA breaks [٥٠]. The consequence of DNA break causes cells death if cells fail to repair damage.

The activation of DNA repair enzyme is as the activation of poly (ADP-ribos) synthetase, deplete the NAD^+ leveles in cells, inhibiting proinsulin synthesis and causing cells to be more sensitive to free radicals[٥٠]. To increase cellular NAD^+ level,it must be supplemented with NAD^+ and Na. This supplementation elevates the efficiency of proinsulin level[٥١].

(٢)Cytokines are released by T cell, Marcophages, and NK cells in the insulitis induce the formation of intracellular free radical causing selective damage to B-cell[٥٢]. The major factor in the damage of Beta cells is interleukin ١ (I L-١) with other factors such as interferon γ (ITF) and tumor necrosis factor (TNF) release macrophages during the insulitis[٥١]. Two types of free radicals are induced from endo theliat cells and beta cells. One type is superoxide. The toxicity of superoxide with hydroxyl radicals could be the same as above. IL-١ can induce the production of nitric oxide synthtase (NOS), the enzyme which is responsible

for the synthesis of $NO^{\cdot}$ (the second type). Many evidences suggested that NO results in directly inhibitory effect on beta cell mitocondrial function [٥٢]. That means $NO^{\cdot}$ can directly destroy beta cell.

(١-٩) Free Radicals in Pathogenesis of NIDDM

The etiology of NIDDM has far more diversity than IDDM [٥٣]. There are many factors, which may induce beta cells malfunction and / or insulin resistance such as genetic abnormalities, environmental factors or obesity. These factors can cause mild hyperglycemic and cause alternations in free radicals generation and antioxidant enzyme [٥٤].

(١-١٠) Creatine Kinase (CK, EC ٢.٧.٣.٢)

Creatine Kinase is a key enzyme of cellular energy metabolism [٥٥], catalyses the reversible transfer of the high energy N-phosphoryl group from creatine phosphate to ADP [٥٥,٥٦,٥٧]. See equation (A) and Fig (١-٩). Three cytosolic (*MM-CK*, *MB-CK*, *BB-CK*) and two mitocondrial isoenzyme (Ubiquitous *Mia-CK* and sarcomerc *Mio-CK*) of CK enzyme are known [٥٨,٥٩].

(١-١٠-١)Clinical Significance

١-Diseases skeletal muscle :-In all types of musculardystrophy ,and chiefly in Duchenn type (in which levels up to ٥٠ times the higher limit of normal may be

encountered) serum CK activity is greaterly elevated at some time during the course of the disease . About ٥٠٪ to ٨٠٪ of the asymptumtic female carries of Duchenne dystrophy show three-to-six fold elevations of CK activity ,but values may be normal following a period of physical inactivity [٧].

٢-Disease of the heart :- After a *myocardial infraction* CK activity iserum is always elevated. The use of total CK and MB-CK in the diagnosis of *myocardial infraction* is the most important single appliance of CK measurement in clinical chemistry and is discussed more fully later .Myocardial damage is usually excluded if an increased MB-CK activity is <٥ %or ٦٪ of the total CK activity[٧].

٣-Disease of the central nervous system :- Serum CK activity may increase after cerebral ischemia , acute cerebrovascular disease ,neurosurgical interventions ,and head injury .In *Reye's syndrome* (a childhood disorder distinguish by acute brain swelling with fatty infiltration and nonicteric dysfunction of the liver) there may be up to a٧٠-fold increase in serum CK activity , representative the severity of the encephalopathy .

٤- Disease of the thyroid :- Serum CK activity demonstrated an inverse relationship with thyroid hormone activity . About ٦٠ % of hypothyroid subjects show an average elevation of CK activity of five times the upper reference limit. The major isoenzyme present is MM-CK ,although up to ١٣ % of CK activity may be present as MB-CK ,suggesting possible myocardial involvement. Hypothyrodism is known to predispose a patient to ischemic heart disease .By contrast ,in *hyberthyroid* subject ,the serum CK activity tends to be at the low end of the reference interval [٧].

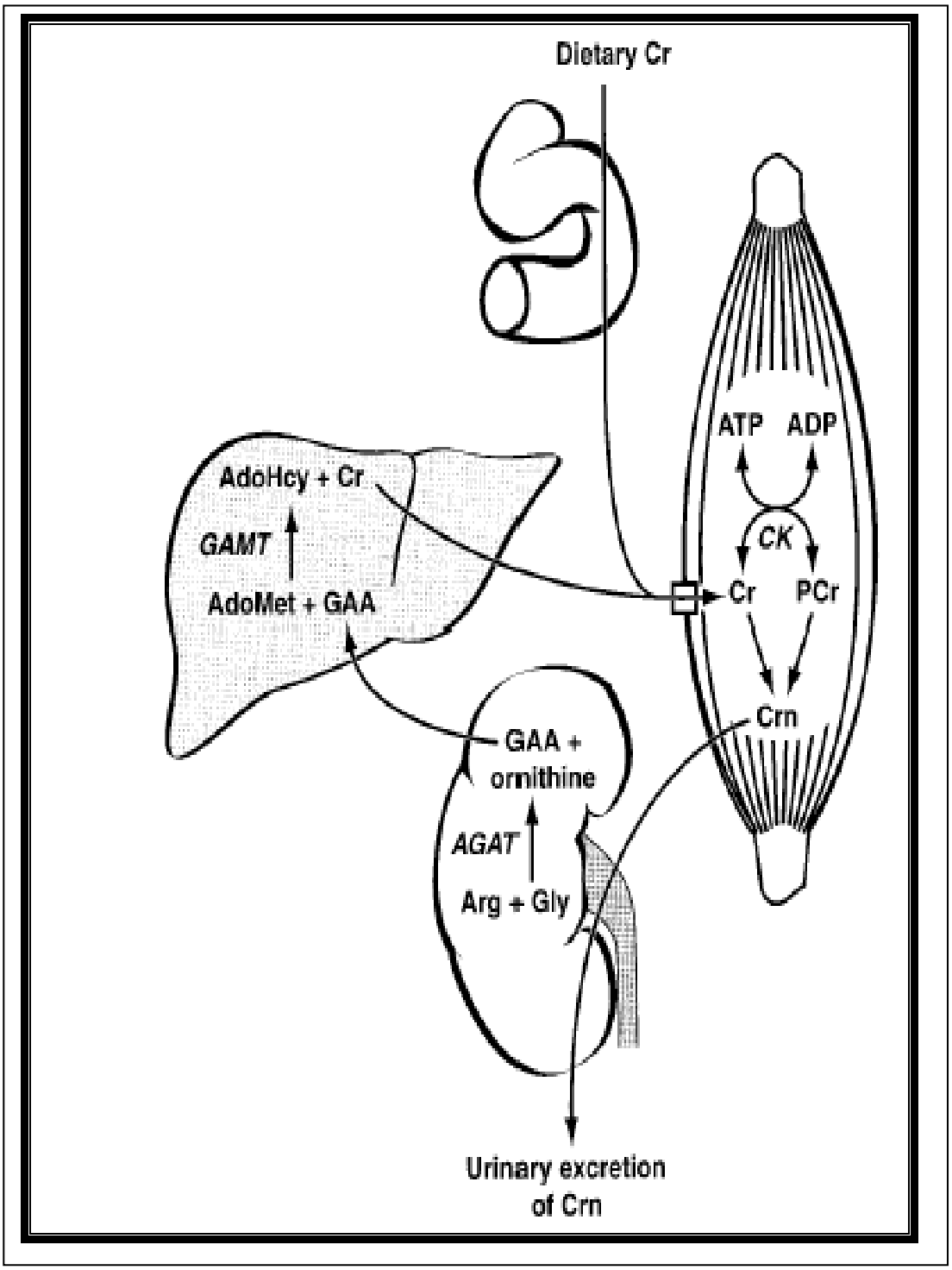
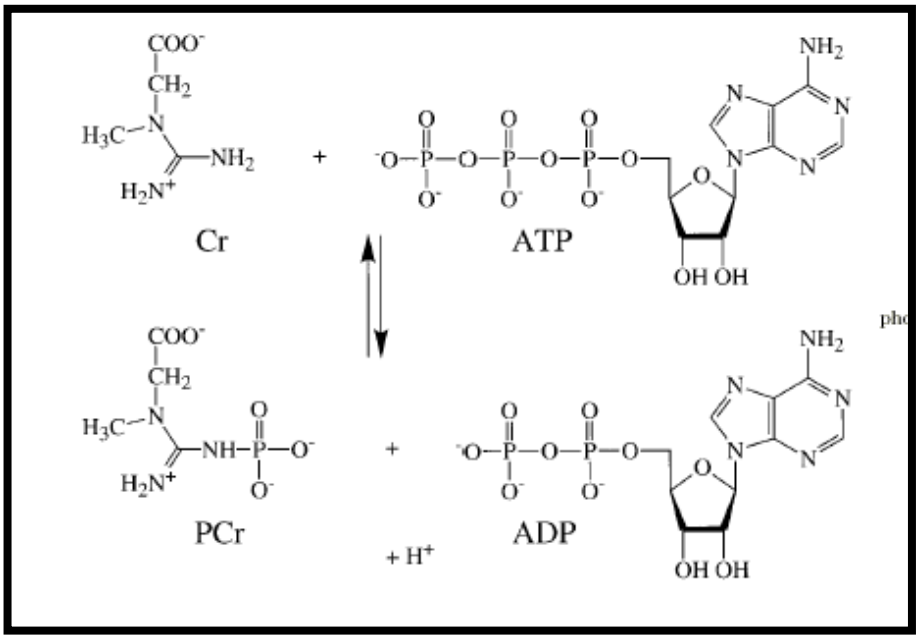


Fig (١-٩): -Major routes of Cr metabolism in the mammalians body .The most part(up to ٩٠ ٪(of Cr is found in muscular tissues(with apology from[٨٤].



Equation (A): CK Function

The mitocondrial isoenzyme (*Mi-CK*) are located in the mitocondrial inter membrane space [٥٦] and are associated with the outer side of the inner membrane [٦٠]. *Mi-CK* differs from its cytosolic counterparts by the ability to from octamer from arrangement of four dimmers to produce stable building block [٦١]. See fig (١-١٠).On the other hand, cytosolic enzymes have strictly dimmeric structure. The *Mi-CK* enzyme gives a cube-like structure with identical top and bottom faces that appear suitable to interact with two-mitocondria member's [٦٢].

A series of experimental findings supporte this structural consideration. Rojo *et al* [٦٣] and Stachowaik *et al* [٦٤] explain *in vitro* that Octameric form of *Mi-CK* was able to connect two artificial membranes. Kottke *et al*[٦٥] observed that mainly octameric form of the enzyme was present in the isolated fractions brain of mitocondria.

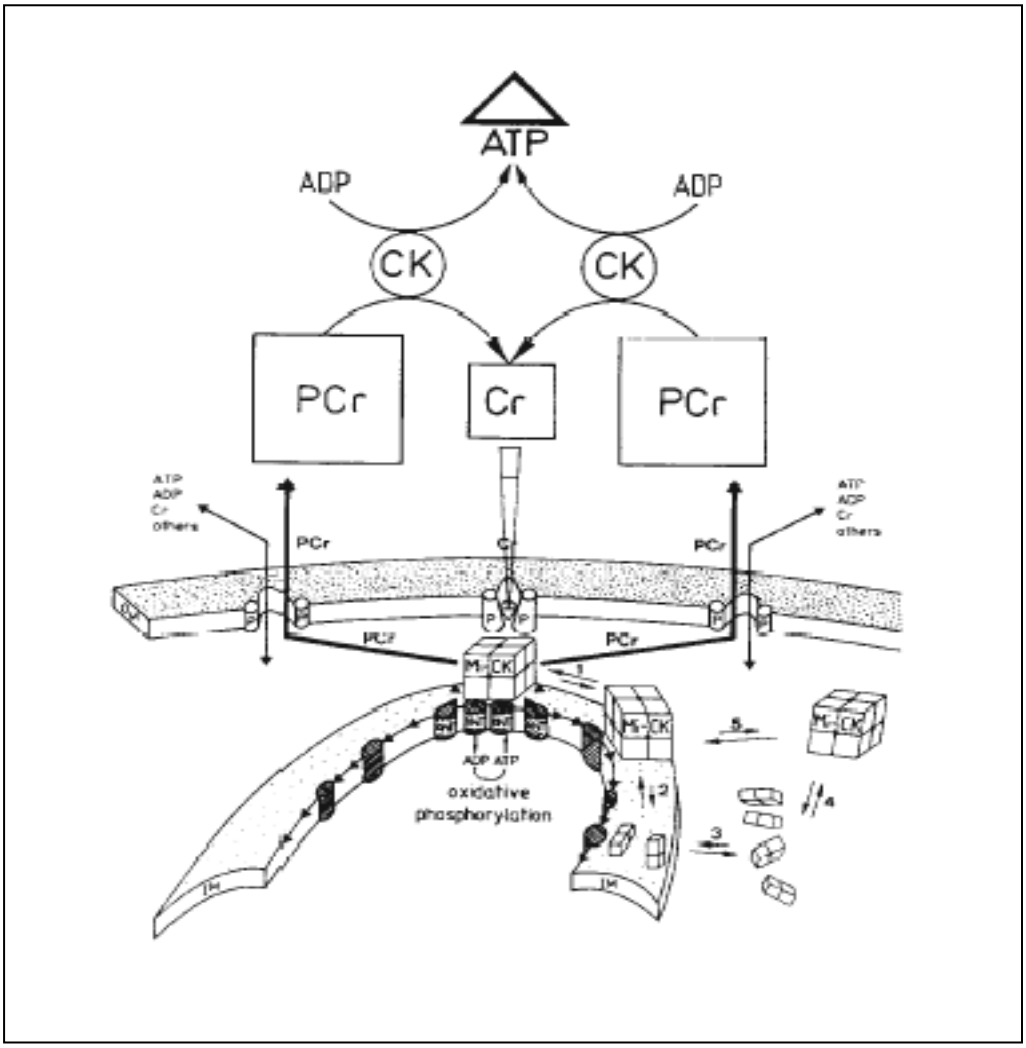


Fig (١-١٠) Model for the possible regulation of CK activity and transport of (high-energy) compounds through the mitocondrial membranes.
((with apology from [٦١]))

Ever since the discovery of the creatine kinase reaction in ١٩٣٤[٦٦,٦٧], research efforts focused mainly on biochemical and physiological properties of CK reaction itself and on "high-energy phosphate" metabolism of cells and tissues with high-energy demands. Fast- twitch skeletal must has a large pool of CrP for regeneration of ATP hydrolyzed during a short period of intense work. The high activity of CK in muscle causing CK- reaction remains in a near-

equilibrium state and keeps [ADP] and [ATP] at constant concentration (over several seconds) [٥٧].

CK isoenzymes are associated with site of ATP production (e.g. Mi-CK in the mitocondrial intermembrane space) and with ATP consumption (e.g. cytosolic CK bond to the myofibrillar M line or the plasma membrane) and fulfill the function of transport demand of high-energy phosphate [٦٨,٦٩].

Lately, the molecular structures of all (CK) isoenzymes have been solved [٧٠,٧١]. The catalytic mechanism of CK stunted by using different techniques that produce detailed information about kinetic and mechanistic aspects of the transphosphorylation reaction [٧٢].

γ- Phosphoryl group is transferred via an associative mechanism [٧٣,٧٤]. At pH ٨ and above. CK reaction follows a rapid equilibrium random mechanism in both directions [٧٥], where as at pH ٧, the kinetic mechanism is random only in the reverse direction (ATP synthesis) and equilibrium ordered with ATP added before Cr, in the forward direction (CrP synthesis) [٧٦].

The information about specific amino acid residues involved in substrate binding and catalysis is scarce. Especially the guanidine substrate-binding site of CK has not yet characterized of the lack specific mutants or a CK crystal structure containing creatine.

The essential cause to decrease the enzymatic activity to CK enzyme is the mutation of the highly reactive cysteine ٢٧٨ and tryptophan ٢٢٣ located near the active site [٧٧,٧٨]. (See fig ١-١١) [٧٣]

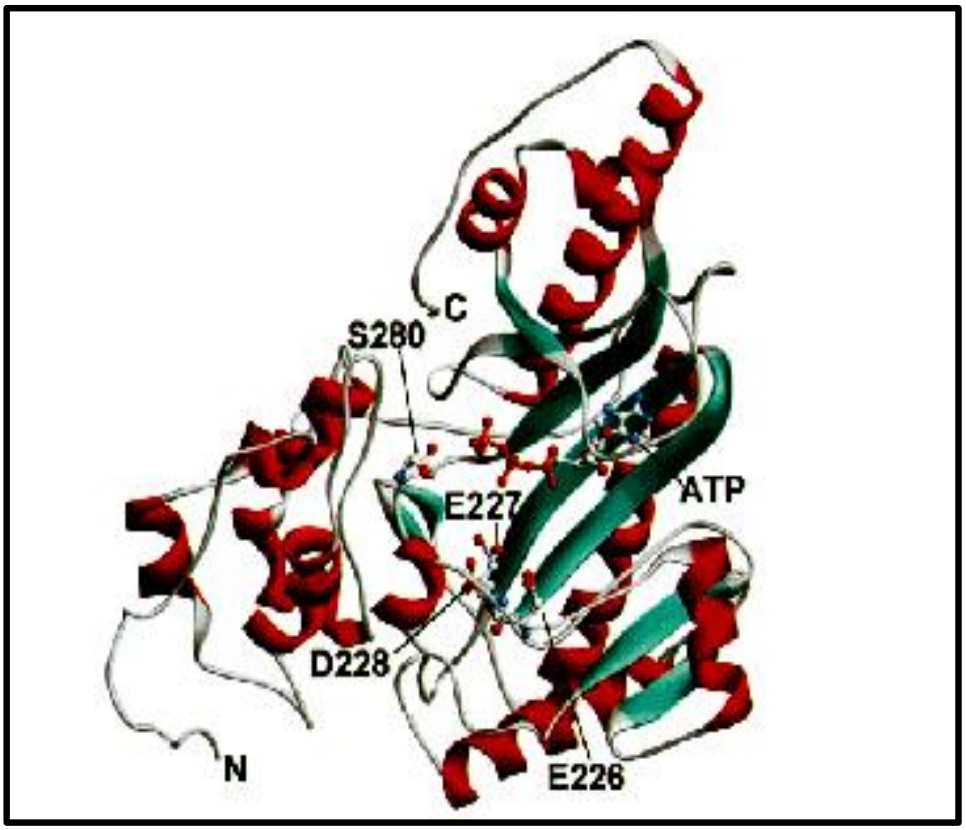


Fig (١-١١): -Location of mutated residues in CK structure. Ribbon representation of three-dimensional fold CK monomer, ATP that marks the Active site of CK, and amino acid mutated in (Glu٢٢٦, Glu٢٢٧, ASP٢٢٨ and Ser٢٨٠) (with apology from [٧٩])

(١-١٠-١) Creatine Kinase and Oxygen Stress: -

There are several evidences, which suggest that CK inhibition occurs because reactive oxygen species is found [(٧٥).] This evidences include (١) MM-CK which beyond to bovine, rabbit and rat heart as well as Mi-CK were inactivated by incubation with either (hypo) xanthine plus xanthine oxidase or hydrogen peroxide with concomitant loss of free sulfhydryl group.

Superoxide dismutase, catalase, reduced glutathione and cysteine are protected against inactivation [٨٠,٨١,٨٢].

(٢) Postischemic repercussion in isolated rat hearts causes decrease in total CKactivity, addition SOD to perfusion medium prevents this effect [٨٣].

(٣) In permeabilized muscle fiber of the rat heart, myofibrillar MM-CK was identified as the primary target of both xanthine oxidase / xanthine and hydrogen peroxide. Inhibition of CK in this state is prevented by catalase or dithiothretol [٨٤].

Oxidative stress induces the release of iron from stored proteins, that makes availability of iron for catalysis of free radical reactions. In fact, ferrous iron catalyze the inactivation of rabbit MM-CK by H_2O_2 or xanthine oxidase / hypoxanthine [٨٦,٨٥]. The micromolars concentrations of iron and iron chelates that were reduced and recycled by superoxide radicals were effective catalyses of CK [٨٧]. Korge and Campbell obtained evidence that iron may directly inhibit CK [٨٨,٨٥]. Inactivation of CK depends on the modification of sulfhydryl group of CK and was prevented by dithiothreitol, desferrioxamine and EDTA.

NO and peroxynitrite were found to inhibit CK reversibly and irreversibly, respectively, most probably by binding to the reactive sulfhydryl group of the enzyme [٨٩,٩٠].

(١-١١) Glutathione

Glutathione is a major intracellular peptide sulfhydryl compound. Glutathione has many biological functions such as maintenance of membrane protein sulfhydryl groups in its reduced form and it functions in catalysis, metabolism, transport and in the protection of cells against foreign compounds, free radicals and ROS [٩١]. Glutathione is an active compound in reactions that

destroy H_2O_2 and other peroxides [٩١]. Glutathione acts also as a cofactor for many enzymes such as glutathione peroxidase, which catalyzes detoxification of intracellular. Thus, it maintains of glutathione levels in its natural state for cellular defense against oxidative injury and for cellular integrity [٩١]. The physiological role of glutathione as an antioxidant described and substantiated in studies of numerous disorders reflecting the increased oxidation result of abnormal glutathione [٩٢].

Glutathione is synthesized interacellularly by consecutive action of γ-glutamylcysteine synthetase (equation-١-) and GSH synthetase (equation-٢-)

$$L-glutamate+L-cysteine+ATP\rightarrow L-glutamyl-L-cystaine+ADP+Pi \quad (1)$$

$$L-glutamyl-L-cysteine+glycine+ATP\rightarrow glutathione+ADP+Pi \quad (2)$$

The synthesis of Glutathione depends on the viability of substrates, cysteine is the limiting substrate. γ- Glutamylcysteine synthetase is non- – allostrically,feed back inhibited by GSH[٢٦]Thats mean under Physiological condition, γ- glutamylcysteine synthetase does probably not act at maximal rate. simple transport of GSH as shown in Fig (١ –١٢)

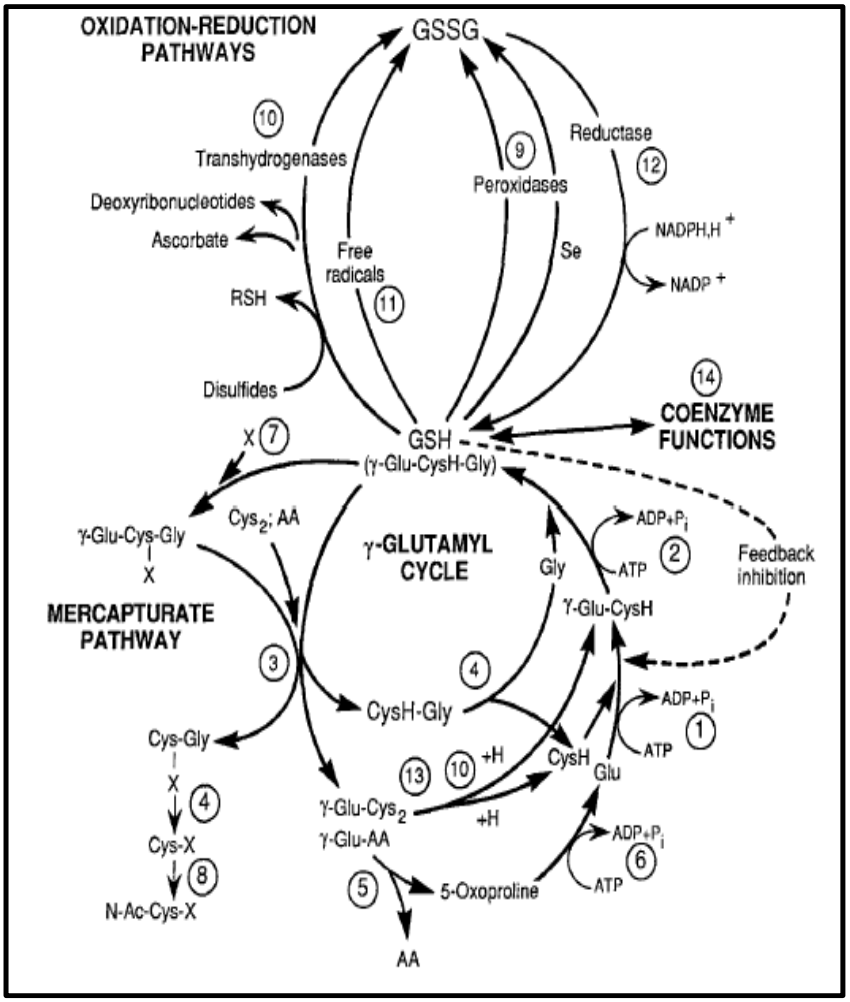


Fig (١-١٢) GSH transport and metabolism in cytoplasm(with apology (from [٩٤])

The depletion of GSH is a very important factor to some serious human diseases, such as chronic renal failure, diabetes, alcoholism and contract formation [٩٣]. These diseases associated with oxidative stress. Glutathione and thiol redox status regulates gene transcription, which participates in the pathogenesis of cancer, diabetes and atherosclerosis [٩٥].

(١-١١- ١) Basic Oxidation– Reduction Reaction :

Thiol reacts as reducing agent, which make GSH easily donate a hydrogen atom. Thiol groups are very reactive towards free oxidizing radicals. The hydrogen atom abstraction is one of the most important property reactivity considering its function as antioxidant .

١) One– electron process: - In this process, GSH undergoes oxidation reaction with free radicals $(\dot{R})$ to produce a sulfur –centered glutathyl radical. $(G\dot{S})$

$$GSH + \dot{R} \rightarrow G\dot{S} + RH \qquad (1)$$

Sulfur– centered glutathyl radicals can also be formed through photoionization of glutathione (Reaction ٢) or through electron transfer to metal ion (Reaction ٣).

$$G\bar{S} \rightarrow G\dot{S} + \bar{e} \quad (2)$$

$$G\bar{S} + \overset{n+}{M} \rightarrow G\dot{S} + \overset{(n-1)+}{M} \quad (3)$$

٢) Two-electron process: - Glutathione anion can be oxidized to produce glutathione cation $G\bar{S}$) (reaction ٤) glutathione cation and glutathione anion converted to glutathione disulfide via oxidation– reduction reaction (reaction ٥)

$$G\bar{S} \rightarrow GSOH \quad (4)$$

$$G\bar{S} + GSOH \rightarrow GSSG + \bar{O}H \quad (5)$$

٣) Glutathione as acosubstrate of glutathione peroxidase- :

Glutathione can decrease free radical production via reducing lipid hydroperoxides of GSH to GSSG, reduce ROOH to alcohol (ROH) (equation –٦) and reduce H_2O_2 to water (equation- ٧)

$$2GSH + ROOH \xrightarrow{GRx} GSSG + ROH + H_2O \quad (6)$$

$$2GSH + H_2O_2 \rightarrow GSSG + 2H_2O \quad (7)$$

There are two types of GPx, the first selenium- dependent GPx, which can catalyze the decomposition of both lipid hydroperoxide and H_2O_2. GPx is made of four protein subunits; each of this protein contains one atom of selenium at the active site. GSH reduces the selenium atom and the reduced form of enzyme.

After reduction, enzyme (GPx) reacts with hydroperoxides or hydrogen peroxide [٩٨]. The other type is named phospholipide hydroperoxide (PhGPx), which also selenium dependent (phGPx). This type acts on membrane to bind ROOH and cholesterol-OOH [٩٨].

٤) Glutathione as a cosubstrate of dehydroascorbate reductase: -

GSH participates in the reduction of dehyroascorbates, which is a well-known antioxidant .

$$dehydroascorbate + 2GSH \rightarrow GSSG + ascorbate \quad (8)$$

To maintain the balance between GSH and GSSG, GSSG is reduced to GSH by glutathione reductase, which uses NADPH as a cofactor .

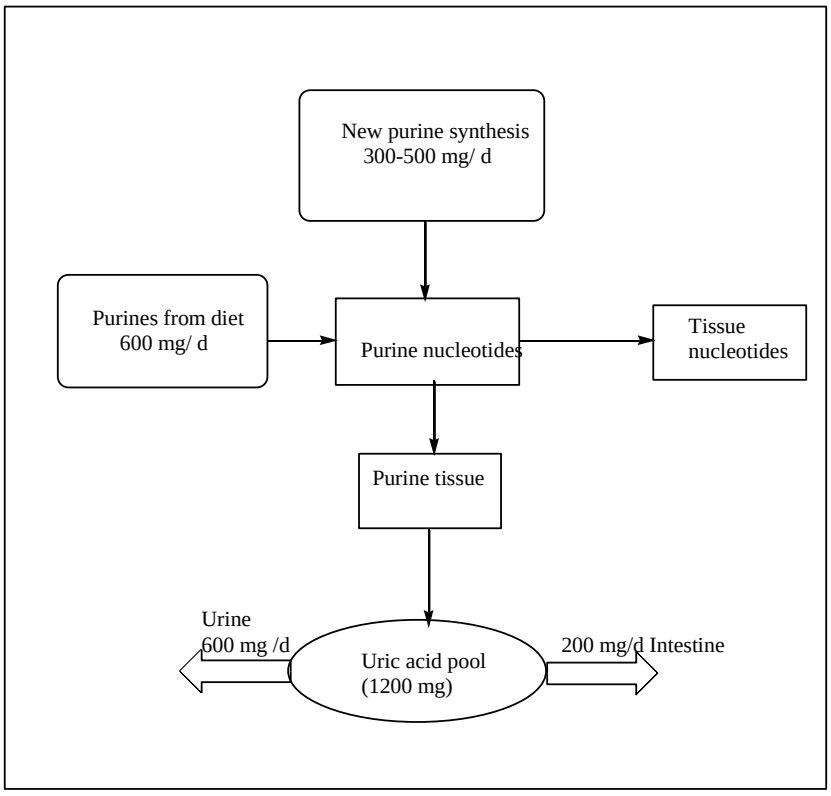
$$GSSG + NADPH + H^+ \rightarrow 2GSH + NADP^+ \quad (9)$$

٥) Glutathione as a direct free radical scavenger - :

In ١٩٩٣, Buettner summarized the reduction potentials of selected set of available antioxidants and free radicals. GSH one of these antioxidants can act as a direct scavenger of almost all of noxious free radical, such as $HO^{\bullet}, O_2^{\bullet -}$ and other carbon-, oxygen-, and nitrogen-centered free radicals, and it is converted into the relatively stable. $G\dot{S}$

(١-١٢) Uric Acid:

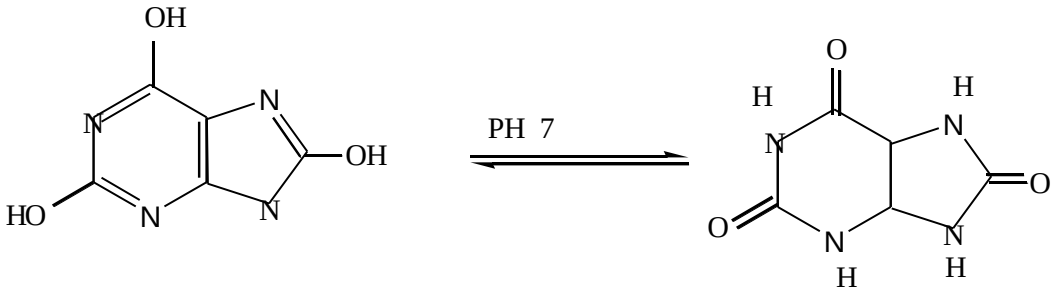
Uric acid (UA) has free radical scavenging properties and known to increase serum antioxidant capacity in its normal value [٩٩]. This compound is present in plasma in relatively high concentrations in women (٣٣٠ μmol/l), in men (٣٨٠ μmol/l) [١٠٠]. Human's body excretes the eccess of uric acid by kidney. The rate of synthesis of uric is equal to the rate of it is excretion and use in biological process. Fig (١-١٣) [١٠٠].



Figure(١-١٣)Synthesis and consumption of uric acid (with apology from [١٠٠])

The increase of the concentration of uric acid causes gout. Uric acid has a lot of beneficial functions in our body. It is shown to be a very important endogenous antioxidant.

Uric acid found in two tautumeric forms [١٠١]:

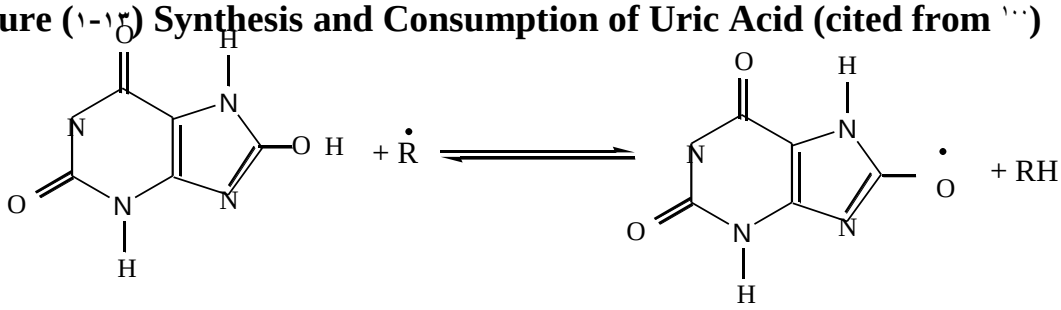


Lactim form Lactam form

(١-١٢-١) Antioxidant Properties of Uric Acid:

١- The reactions of uric acid with alkyl radicals [١٠٠].

Figure (١-١٣) Synthesis and Consumption of Uric Acid (cited from [١٠١])



٢ -The reaction of uric acid with peroxy radicals.

$$RO\dot{O} + UH_2^- \rightarrow ROO^- + UH^{\bullet -} + H^+$$

٣ -Reaction with ascorbate: -

The redox potential of uric acid (E = ٠.٥,at pH٧ (more than that of ascorbic acid (٠.٢٨ at pH٧ .(U.A was shown to donate electron to ascorbate and thus prevented it is deleterious on some enzyme [١٠٠].

$$UH^{\bullet -} + AsCH^{-} \rightarrow UH_2^{-} + AsCH^{\bullet -}$$

٤-peroxinitrite is one of the most important reactive species in human body [١٠٣], as show in fig (١-١٥) [١٠٢]

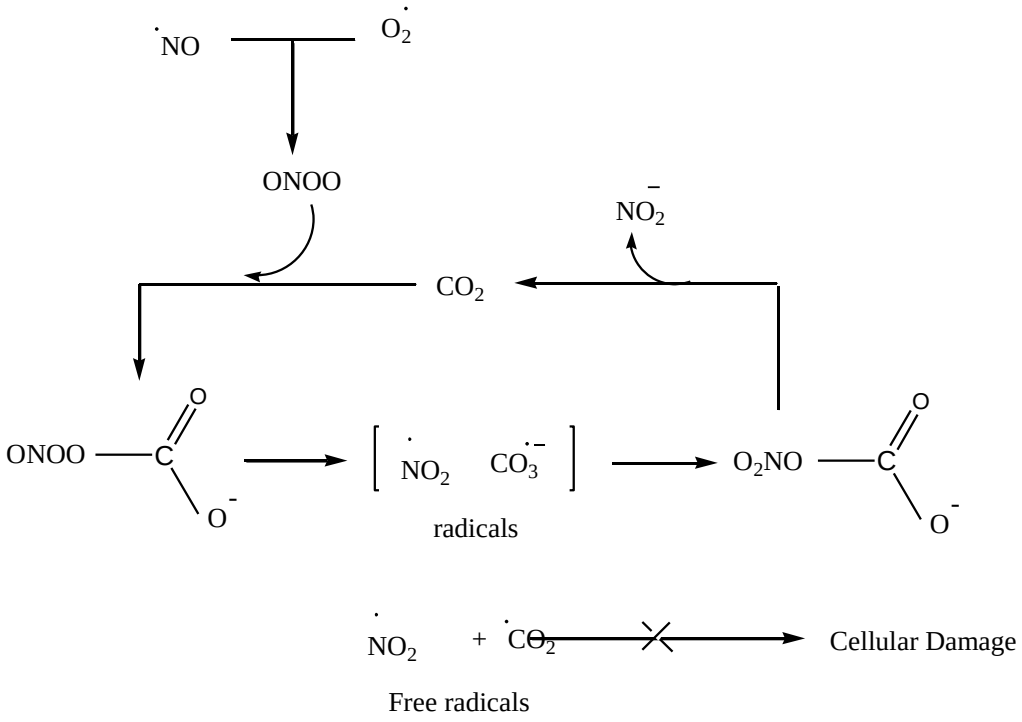


Fig (١-١٥) Peroxynitrate synthesis ((cited from [١٠٢]))

٥-Reaction with $NO_2^{\bullet}$: -

Uric acid reacts with $\dot{N}O_2$ and inactivates it

$NO^{\cdot -} + UH^{2-} \longrightarrow NO^{2-} + UH^{-}$

٦-Detection of uric acid

Uric acid can be detected by using the test which depends upon the formation of murexide (an ammonium salt), which is an intense reddish purple. Nitric oxide is added to the urine, which is then evaporated. If uric acid is present, muroxide is formed after adding ammonia to the residue. Fig (١-١٥) [١٠٠]

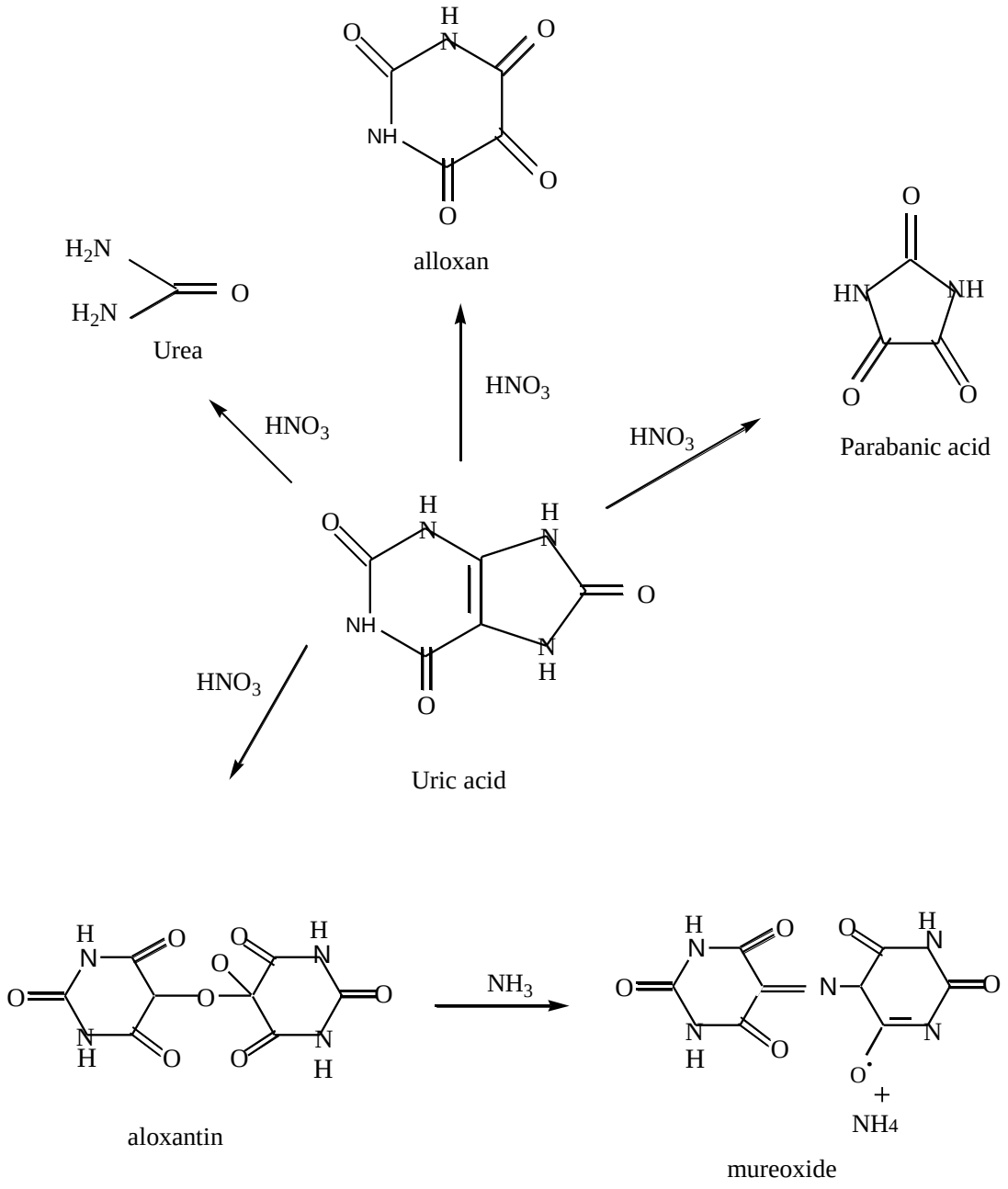


Fig (١-١٦) Detection of uric acid ((with apology from[١٠٠]))

(١-١٣) Lipid Profiles and Oxidation Hypothesis:

Fatty acids (R-COOH) are straight– chain compounds, which have varying length ,may be saturated or unsaturated . In the blood, fatty acids are carried mainly in conjugated form (bound to albumin) .There are four types of the lipids present in plasma, cholesterol, cholesterol ester, triglyceride and phospholipids. These lipids are relatively insoluble in water but are carried in the body fluids as soluble protein complexes known lipoproteins [١٠٣,١٠٤].

(١-١٣-١) Cholesterol:

It has a steroid structure, and found in plasma either as esterified form with fatty acids forming cholesterol ester or as free cholesterol [١٠٣,١٠٥].

In patients with diabetes ,cholesterol metabolism differs from non-diabetes patents because cholesterol synthesis is high [١٠٦,١٠٧,١٠٨] and reduced by insulin [١٠٩].

(١-١٣-١-١) Cholesterol hydroperoxides generation.

Lipid hydroperoxides (LOOHs) can be generated when cholesterol (Ch)and other unsaturated fatty acids in cell membranes are degraded under conditions associated with oxidative stress [١١٠]. LOOHs are more polar than parent lipids and much longer than free radical precursors and products [١١١,١١٢].

Propagative (chain) cholesterol hydroperoxidation can be triggered by a wide variety of chemical agents. Equation (١-٧) explains this reaction [١١٠], where ٥α-OOH is depicted as triggering ChOOH.

$$5\alpha - OOH + Fe^{+2} \rightarrow 5\alpha - O^{\bullet} + OH^{-} + Fe^{+3} \quad (1)$$

$$Ch + 5\alpha - O^{\bullet} \rightarrow Ch^{\bullet} + 5\alpha - OH \quad (2)$$

$$Ch^{\bullet} + O_2 \rightarrow 7\alpha\beta - OO^{\bullet} \quad (3)$$

$$7\alpha\beta - OO^{\bullet} + Ch \rightarrow 7\alpha\beta - OOH + Ch^{\bullet} \quad (4)$$

$$7\alpha\beta - OO^{\bullet} + Ch \rightarrow 7\alpha\beta - O^{\bullet} + 5,6epoxide \quad (5)$$

$$7\alpha\beta - OOH + Fe^{+2} \rightarrow 7\alpha\beta - O^{\bullet} + OH^{-} + Fe^{+3} \quad (6)$$

$$7\alpha\beta - O^{\bullet} + Ch \rightarrow 7\alpha\beta - OH + Ch^{\bullet} \quad (7)$$

(١-١٣-٢) Triglycerdes

Consists of glycerol, and each molecule of which is esterfied with three fatty acids [١٠٣,١٠٥].

١-١٣-٣ Phospholipids:

Compound are found as triglycerides but contain phosphate and nitrogenous base instead of the third fatty acid molecule at carbon three in glycerol. The phosphate and nitrogenous base are water-soluble which play a role in lipid transport [١٠٣,١٠٥].

(١-١٣-٤) Lipoproteins:

There are four types of lipoprotein, which vary in their size, function and composition. The most important lipoproteins for cholesterol transport are: -

a- High-density lipoprotein (HDL (which transports cholesterol from liver to cell).

b- Low density lipoprotein (LDL) which transports cholesterol from cells to liver.

The most important lipoprotein for triglyceride transport is- :

١- Very low density lipoproteins (vLDL), which transport endogenous triglycerides from liver to cell.

٢- Chylomicrons transport exogenous (dietary) triglycerides from the gut.

A transient intermediate in the metabolism of very low-density lipoprotein to low density lipoprotein is termed intermediate density lipoprotein (IDL). It contains both cholesterol and endogenous triglycerides and in normal persons is undetectable in plasma [١١٣,١١٤]. The constituents of of these lipoproteins and their densities are shown in table (١-٢).

Table (١-٢): The constituent of lipoproteins in plasma of healthy human's [١١٤].

Lipoprotein	Protein	Total lipid	Cholesterol	Phospholipid	Triglyceride
	%	%	%	%	%
Chylomicron	١	٩٩	٤	٧	٨٨
(vLDL)	٨	٩٢	٢٢	١٦	٥٤
(LDL)	٢١	٧٩	٤٦	٢٢	١١
(Lpa)	٣٦	٦٤	٣٧	١٨	٩
(HDL)	٥٠	٥٠	٢٠	٢٦	٤
Free fatty acid-albumin	٩٩	١	٠	٠	٠

Study the amount of lipoprotein helps to indicate other diseases that is associated with diabetes such as a therosclerosis [١١٥], myotonic dystrophy [١١٦] and cardiovascular disease (CVD)[١١٧,١١٨,١١٩].

(١-١٤) Lipid Peroxidation

Poly unsaturated fatty acid (PUFA) are very important components of cell membrane. They form a double layer structure of membrane. They are arranged to be the non-polar tail faces towards the inner side of the double layer structure [١٢٠]. Oxidant can react with polyunsaturated fatty acid in cell membrane to form toxic metabolites. Lipids, which contain two or more unsaturated carbon-carbon double bond (C=C) can be attached by reactive oxygen species (ROS) Fig (١-١٧) [١٢١]

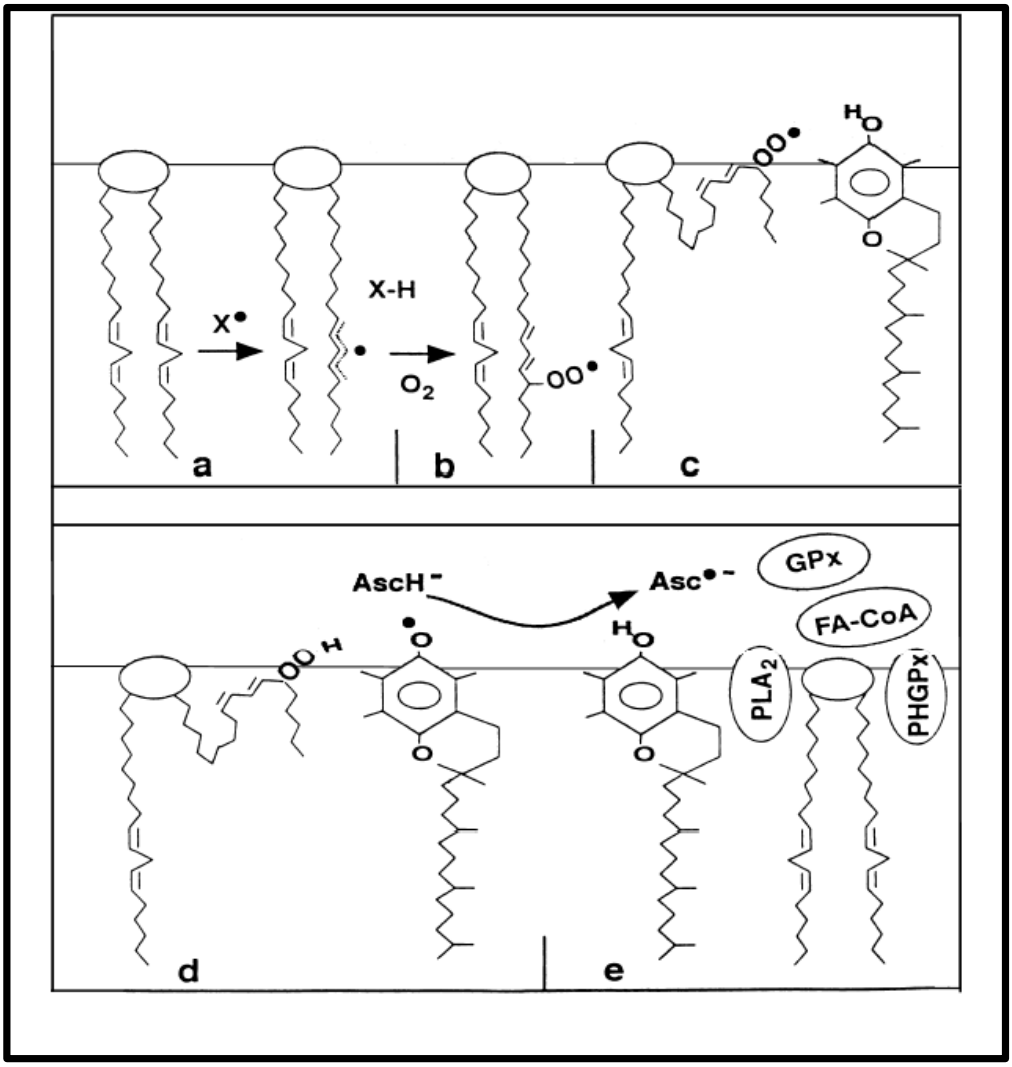


Figure (١-١٧) Membrane lipid peroxidation. (a) Initiation of the peroxidation process by an oxidizing radical $X^{\bullet}$, by abstraction of a hydrogen atom, thereby forming a pentadienyl radical. (b) Oxygenation to form a peroxyl radical and conjugated diene. (c) Peroxyl radical moiety partitions to the water–membrane interface where it is poised for repair by tocopherol. (d) Peroxyl radical is covered to a lipid hydroperoxide, and the resulting tocopherol radical can be repaired by ascorbate. (e) Tocopherol has been recycled by ascorbate; the resulting ascorbate radical can be recycled by enzyme systems. The enzymes phosphlipase A٢ (PLA٢), phosphlipid hydroperoxide glutathione peroxidase (PH-GPx), glutathione peroxidase (GPx) and fatty acyl-coenzyme A (FA-CoA) cooperate to detoxify and repair the oxidized fatty acid chain of the phosphlipid.

(١-١٤-١) Formation of $L\dot{O}O$

Initiation: - Initiation of lipid peroxidation occurs when there are radical species ,such as the hydroxyl radical $(\dot{O}H)$,removes an allylic hydrogen from PUFA producing a lipid radical .$(\dot{L})$ Rearrangement process occurs to get more stable lipid radical ,which is diene or conjugated [١٢٢]. This radical can react with oxygen ((in the presence of oxygen)) giving rise to a lipid peroxyl radical $(L\dot{O}O)$ .Because of highly reactive properties ,hydroxyl radical can react non selectively with unsaturated fatty acids to form a carbon centered radical, either by addition to double bounds or by abstraction of a hydrogen atom from the fatty acid [١٢٣].

$$L-H+\dot{X}\rightarrow\dot{L}+XH$$

Propagation- :

Molecular rearrangement process occurs after forming carbon-centered radicals. Lipid peroxyl radical abstracts an allylic hydrogen atom from another molecule such as adjacent PUFA to form a lipid hydroperoxide (LOOH) and a second lipid radical $(\dot{L})$ .This second lipid radical can behave with the same reactions as the first, generating lipid hydroperoxides. The $(L\dot{O}O)$ radicals are able to subtract a hydrogen atom from another molecule. This causes the lipid peroxidation. The formed carbon radical can further undergo the oxygen addition reaction to form another peroxyl radical and so the chain

reaction of lipid peroxidation continues. The peroxyl radical combines with the hydrogen atom it abstracts giving a lipid hydroperoxide: -

$$LO\dot{O} + L - H \rightarrow LOOH + \dot{L}$$
$$\dot{L} + O_2 \rightarrow LO\dot{O}$$

Termination- :

Termination occurs when any compound acts as free radical trap, forming a stable end product .The hydroperoxide produced may undergo different reactions to terminate the lipid peroxidation process such as that reduced to hydroxyl fatty acid or undergo cyclization to produce cyclic endo peroxides [١٧٤]. Since PUFAs have a number of C-H bonds susceptible to free radical attack, several end products can be generated from PUFA during lipid peroxidation.

$$LO\dot{O} + LO\dot{O} \rightarrow non - radical \quad product$$
$$\dot{L} + LO\dot{O} \rightarrow non - radical \quad product$$
$$\dot{L} + \dot{L} \rightarrow non - radical \quad product$$

Iron in lipid peroxidation- :

LH composing biological membranes can be oxidized to LOOH. The concentration of preexisting LOOH strongly effects the formation of new LOOH [١٧٥]. New LOOH produced by reactive species that have enough energy to break an allylic C-H bond of LH that occurs if the concentration of preexisting LOOH were low. Hydrogen abstraction and lipid alkyl radicals' $(\dot{L})$ formation are involved in this process. When the preexisting LOOH are abundant ,new LOOH will produce highly reactive lipid alkoxyl radicals $(L\dot{O})$ .from the iron- catalyzed decomposition of (LH). After that $(\dot{L})$ radicals are formed by hydrogen abstraction from LH. In both cases, Fe (II) is required.

$$LOOH + Fe(II) \rightarrow L\dot{O} + Fe(III)$$

Ferritin, transferrin and lactoferrin are possible sources of Fe (II)[١٢٦], because these protein (called iron –binding protein) have higher affinity for Fe (III) than that for Fe (II). Upon suitable reductance, they release iron in a form of Fe (II)[١٢٧]. This suitable reductance may be super oxide [١٢٨] $\dot{O}_2^-$ and ascorbat [١٢٩].

(١-١٤-٢) Removal of Lipid Peroxyl Radicals:

There is a number of ways to protect the cell from the threat of radicals produced in lipid peroxidation.

١- Enzymatic species act to minimize the detrimental effect of free radicals such as superoxide dismutase, glutathione peroxidase and catalase [١٣٠].

٢-By numerous removal and repair enzymes such as phospholipase $A_٢$, which is available to repair the damaged molecule [١٣١].

٣-Non-enzymatic antioxidant compound such as vitamine E (alpha- tocopherol) and vitamine C (ascrobic acid)[١٣٢]. Alpha –tocopherol, a fat-soluble vitamine, is major membrane –bound antioxidant used by the cell. On the other hand, ascorbic acid is regarded as the major aqueous-phase of anti oxidant. Alpha tocopherol and ascorbic acid act together in a cyclic reaction as in Fig (١-١٨) to protect the body from lipid peroxylradical [١٣٣]. The first step in process, alpha tocopherol converts to a peroxyl radical by donating hydrogen to a lipid peroxyl radical. Alpha tocoperol radical is energetically stable and has low reactivity with other molecules within the membrane. The second step, alpha tocopherol is re-reduced to be the original form by ascorbic acid [١٣٣].

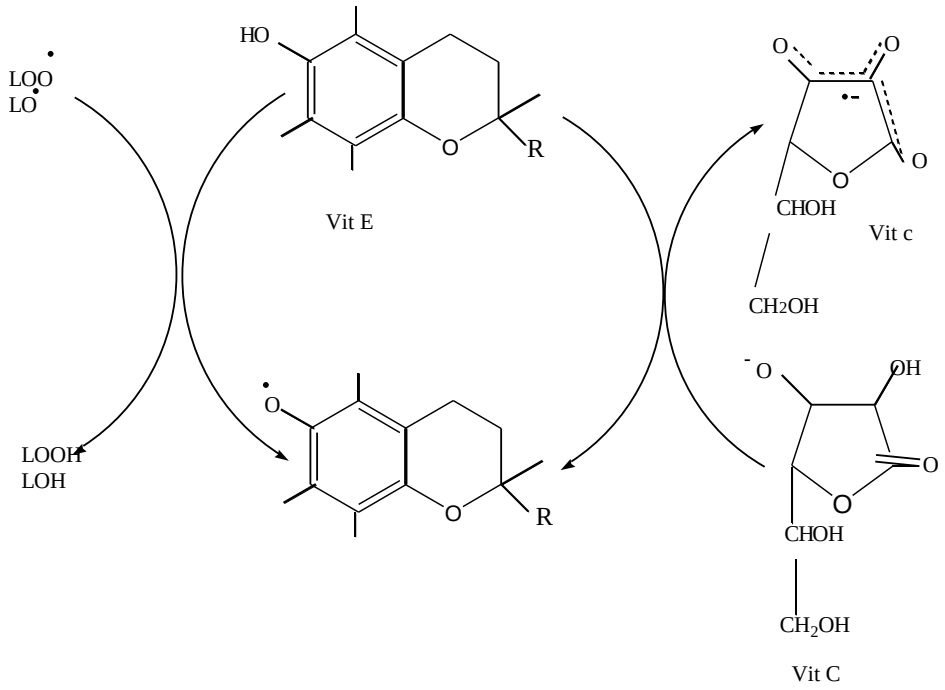


Fig (١-١٨) Chain reaction of vit E and vit C with peroxyl radical [١٣٣]

(١-١٤-٣) Measurement of Lipid Peroxidation

There are many methods available to determine lipid peroxidation such as measuring lipid hydroperoxide or degradation products [١٣٤,١٣٥]. Lipid hydroperoxide can be measured by HPLC following dissolving the hydroperoxide into a polar solvent, which can separate between less polar triacylglecerol and cholesterol hydroperoxide and more polar free fatty acid and phospholipid hydroperoxide [١٣٦]. The most utilized assay for measuring lipid peroxidation is the thiobarbituric acid reactive substance (TBARS) test [١٣٧,١٣٨]. The appearance of (TBAreactivity) varies with the exact concentration of acid, type of acid and period of heating used in the (TBA) assay [١٣٩]). Lipid peroxidation must be indicated to study the effect of oxidative stress on the tissue [١٤٠,١٤١].

(١-١٤) Aim of the Study

١- Investigating the relationship between the CK activity and antioxidant in diabetes mellitus type Iand II.

٢- .Separating the isoenzymes of CK to know which of these isoenzyme influence with oxidative stress in diabetes mellitus.

٣- Determining the oxidative stress associated with diabetes mellitus by measuring the concentration of substances that act as antioxidant such as glutathione and uric acid.

٤- Determining the concentration of lipid profile such as HDL, LDL, VLDL and triglyceride.

٥- Determining the lipid peroxidation by measuring the concentration of MDA.

Chapter Two

٢- Materials

(٢-١-١) Chemicals

All chemicals were use as supplied without further purification.

Chemicals	Purity %	Supplied company
Ethylene diamine tetra acetic dihydrate (EDTA).٢ $H_٢O$	٩٩.٥	Fluka
Methanol	٩٩.٨	Fluka
Ethanol	٩٩.٩٨	Fluka
Tris(hydroxy methylene) amino methane	٩٩.٥	Merck
Glutathione	٩٩.٥	Biochemical
5,5̄-Dithiobis (٢-nitrobenzoic acid)(DTNB)	٩٩.٥	Sigma chemical
Tri chloroacetic	٩٩.٠	Hopkin williams
Thiobarbituricacid(٤.٦Dihydroxy٢-Mercaptopyridine	٩٨.٠	Fluka
Sodium hydroxide	٩٩.٠	Aldrich
Sodium chloride	٩٨.٠	BDH
Ethylen diamine tetra acetic acid di sodiume salt	٩٩.٠	BDH
Creatine kinase kit	----	Randox
uric acid kit	------	Biomagerb kit
Total chloesterol kit	------	Bio merieux
Triglyceride kit	-----	Bio merieux
High density lipo protein kit	-----	Bio merieux
DEAE-cellulose	٩٩.٥	Sigma
Magnesiun actate	٩٩.٥	BDH
Hydrochloric acid	٩٩.٥	Merck

(٢-١-٢) Instrument Analysis and Equipment

Instrument	Supplied company
PH meter	Jenway(Germany)
Sensitive balance	Stanton ٤٦١ AN(Germany)
Vortex mixer	Karlkole (Germany)
Water bath	Karlkole (Germany)
Shaker water bath	Tecam(England)
Oven	Hearson (England)
Magnetic stirrer	Gallin kamp (England)
Spectro photo meter ٢١	Molton roy (switzorland)
Centrifuge	Heraeus (Germany)

(٢-٢) Methodologies

(٢-٢-١) Collection of Blood and Serum Preparation

The vein on the front of elbow or forearm is almost employed. The arm should be warm to improve the circulation and distend the vein. The arm is extended and a tourniquet is firmly applied (١٠-١٥) cm above the elbow. The skin over the vein will be sterilized with a small pad of cotton wool soaked with hapten. Disposable sterile needle fixed on to a disposable syringe is inserted into the vein. When the needle enters the vein the plunger of the syringe is slightly withdrawn. If blood appears, the tourniquet is released. When five ml of blood has been drawn into the needle; then, the needle is withdrawn. This pad is firmly pressed onto until the bleeding stops. The needle is removed from the syringe and the blood slowly transferred to plain tube without anticoagulant. The blood allowed to clot for ١٥ minutes, the clot shrinks and serum can be obtained by centrifuging for approximately ١٠ minutes at a relative centrifugal force (RCF) of ١٠٠٠ xg to ٢٠٠٠ xg[٣٩,١٦٧,١٦٨].

(٢-٢-٢) Patients and Controls

Thirty patients (٢١ males, ٩ female) with diabetes mellitus type I and twenty-four (١٥ males, ٩ females) with diabetes mellitus type II has been subjected to the present study, as well as fifty apparently healthy individuals as a control (٣١ males, ١٩ females) after having been asked about their health. (All the samples is collected from The laboratories of Margan hospital in Hilla city)

(٢-٢-٣) Determination Serum Creatine Kinase Activity

In the present study, the creatine kinse activity was measured by using commercially available kits (randox laboratories Ltd., UK)[١٤٢,١٤٣,١٤٤].

(٢-٢-٣-١) Principle

Creatine kinase (CK) utilizes creatine phosphate as substrate to act as the initial catalyst for a series of reactions resulting in the formation of NADPH) as outlined in the coupled enzyme assay shown below. The product from this reaction (NADPH) is proportional to CK activity and used to reduce nitro blue tetrazolium (NBT) in the presence of diaphorase to produce the blue/violet color of diaformazan. This compound has an absorption maximum of around ٥٦٠ nm hydrochloricacid added to stop the reaction

$$creatine\ phosphate + ADP \xrightarrow{CK} creatine + ATP$$
$$Glucose + ATP \xrightarrow{HK} Glucose - 6 - phosphate + ADP$$
$$Glucose - 6 - p + NADP^{+} \xrightarrow{G6p-DH} Gluconate - 6 - p + NADPH$$

CK activity is stable for ٧ days at +٢ to+٨ or ١٤days at-$20^{\circ}C$.

(٢-٢-٣-٢) Reagents

Contents	Concentration in the test
Buffer/Glucose	pH ٦.٤
Imidozol buffer	mol/l
Glucose	٢٠ mmol/l
Mg acetate	١٠ mmol/l
EDTA	٢.٠mmol/l
NBT	٠.٧٣mmol/l
Color reagent	١.١mmol/l
ADP	٤.٠mmol/l
Diadenosine	١٠.٦٣ μmol/l
$NADP^+$	٠.٨mmol/l
Creatine phosphate	٢٥mmol/l
Glutathione	١٢mmol/l
HK	≥ ٢٥٠٠ U/l
G٦P-DH	≥ ١٥٠٠ U/l
Diaphorase	≥ ٢٥٠٠ U/l
Hydrochloric acid	١.٠ N
Standard Creatine kinase	١٨٠ IU/l

(٢-٢-٣-٣) Preparation of Solutions: -

١- buffer/Glucose

Contents ready for use.

٢- Color reagent.

Reconstitute the contents of one vial of the color reagent with exactly ٣ml of buffer/Glucose solution. Stabilize for ٢٤ hours at +٢ to +٨°C or ٨ hours at +١٥ to +٢٥°C.

٣-hydrochloric acid(١ N)

Dilute exactly ten folds with distilled water to give ٠.١ N hydrochloric acid.

٤-standard

Reconstitute with exactly ٥ ml of distilled water.

Dissolve the contents by swirling gently and leave for ٣٠ minutes before use. Once reconstituted, stabilize for ٨ hours at ٢٥° or ٢ days at +٢ to +٨° C.

(٢-٢-٣-٤) Procedure

$wavelength \rightarrow 560nm$

$cuvette \rightarrow 1cm\ path\ length$

$temperature \rightarrow 37\,^{\circ}C$

$measurement \rightarrow Against \quad reagent \quad blank$

	Sample	Standard	Reagent blanck	Sample blanck
Color reagent	٠.٥	٠.٥	٠.٥	٠.٥

solution				
Incubate for ٣ minute at ٣٧ْC				
Sample	٠.٠٥ml (serum)	٠.٠٥ (standard)	٠.٠٥ml distilled	-------
Incubate for ١٠ minute at ٣٧ْC				
٠.١N HCl	٥ml	٥ml	٥ml	٥ml
Sample	---	-----	---	٠.٠٥ml (serum)

The absorbency of the sample (A sample), standard (A stand) and sample blank (A sample blank) are measured against the reagent blankat ٥٦٠ nm.

(٢-٢-٣-٥) Calculation: -

Normal serum: -

$$\text{CK activity in sample} = \frac{\text{A sample}}{\text{A s tan dard}} X \; s\tan ard\ value$$

Highly turbid or icteric serum: -

$$\text{CK activity in sample} = \frac{\text{A sample - A sample blank}}{A \quad s\tan dard} * s\tan dard\ value$$

(٢-٢-٤): -Separation of the serum creatine kinase isoenzyme by ion-exchange column chromatography.

To separate creatine kinase isoenzyme Mercer's method must be used. [١٤٥,١٤٦] This method modified to increase sensitivity by using DEAE-cellulose

rather than DEAE-sephadex-A-٥٠, and by changing the ionic strength of the eluting buffer [١٤٧,١٤٨,١٤٩,١٥٠].

(٢-٢-٤-١) Preparation of DEAE- cellulose

DEAE- cellulose, which has an exchange capacity ٠.٩ mmol/g of dry gel. Two gm from this gel cycled at room temperature through the following sequence of washes: - ٠.٥ mol/l NaOH, water, ٠.٥ mol/l NaOH, water, ٠.٥ mol/l and water. Water washing was continued until the elusion was neutral. Excess liquid was removed by using (Buchner funnel). After the last washing the DEAE- cellulose was washed several times with buffer number-١- (pH ٧.٩), it was kept in this buffer while it was being used.

(٢-٢-٤-٢)Preparing the Buffers

Buffer number-١-

(pH. ٧,٩) ((Composition in mM: ٢٠ NaCl, ٥-magnesium acetate, ٠.٤ EDTA and ١٠٠ tris base))

A-NaCl (٢٠Mm)

٠.١١٦٨ gm of NaCl dissolved in a final volume of ٢٠ deionized water (AL-Furat company).

B-Magnisum acetate (٥ mM)

٠.١٠٧٢ gm dissolved in final volume ٢٠ ml de ionized water.

C-EDTA (٠.٤ mM)

٠.٠١١٦ gm dissolved in final volume ٢٠ ml deionized water.

D-tris (hydroxy methyl) amino methane ((tris) ١٠٠ mM)

١.٢١١٤ gm dissolved in final volume ٢٠ ml deionized water.

E- hydrochloric acid (HCl ١٠٠ Mm)

٠.٦٢٣١ ml of concentrated HCl (sp.gr.١.١٩, ٣٢٪) is added to ١٠ ml of deionized water and brought to a final volume of ٢٠ ml.

A,B,C,D and E, mixed in a volumetric flask (١٠٠ ml), pH adjusted with ١M HCl.

Buffer number-٢-(pH ٦.٤)

Same procedure used to prepare buffer –١-, but differ in concentration of NaCl (٤٠mM, ٠.٢٣٣٧ gm))

Buffer number-٣- (pH٦.٤)

Same procedure used to prepare buffer –١-, but differs in concentration of NaCl ((٢٥٠ mM, ١.٤٦١ gm))

(٢-٢-٤-٣) Procedure

١. Two ml of buffer –١- was allowed to pass through the column.

٢. A١٢٥-mm disposible Pasteur pipette filled with the prepared DEAE-cellulose, after that, translate this gel to column which has an interned dimension (٥x٧٠mm).

٣. Two ml of buffer –١- was allowed to pass throw the column.

٤. One ml of serum was added to the column.

٥.Four mls of buffer-١-were added to elute MM-CK isoenzyme in order to calculate (CK) activity in each one of these four ml_s.

٦. Four mls of buffer-١-were added to elute MB-CK isoenzyme in order to calculate (CK) activity in each one of these four ml.

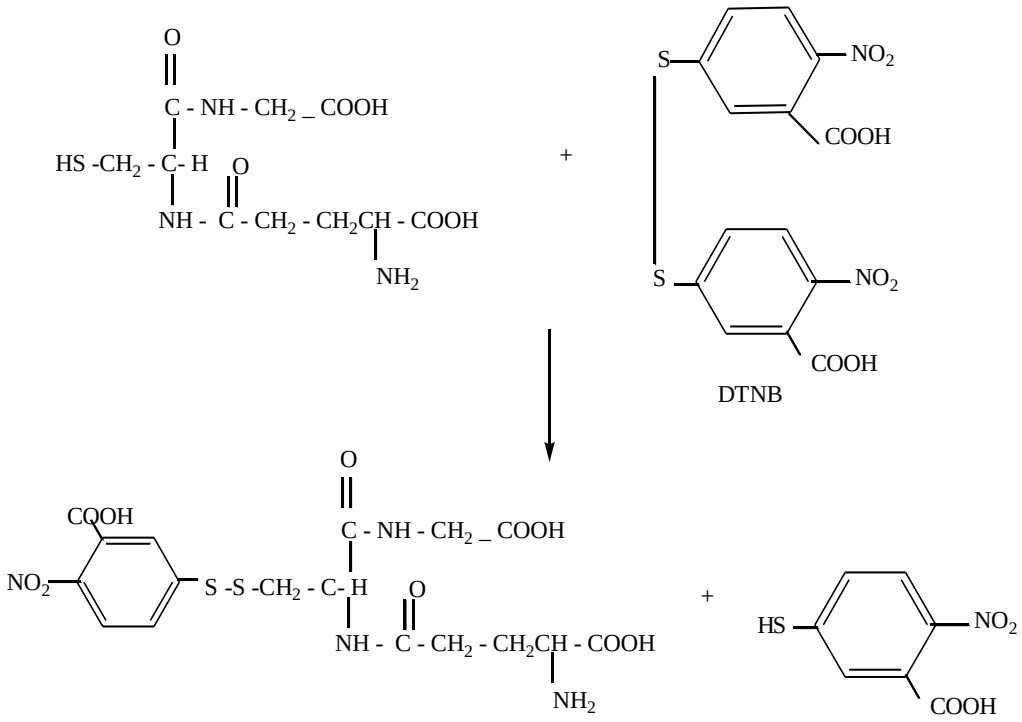
٧. Four mls of buffer-١-were added to elute BB-CK isoenzyme in order to calculate (CK) activity in each one of these four ml.

(٢-٢-٥) Determination of serum reduced glutathione (GSH)

More than one type of analytical methods are used to determine serum glutathione (GSH) depending on the action of sulfhydryl groups. Thus methods include photometric, enzymatic, flourometric and HPLC are used [١٥١,١٥٢,١٥٣].

(٢-٢-٥-١) Principle

5,5̄- Dithiobis (٢-nitrobenzoic acid)(DTNB) is a dichromogen that is readily reduced by sulfhydryl group of GSH to produce an intensely yellow compound. Reduced chromogen has maxmum absorbance at ٤١٢ nm and is directly proportional to GSH concentration. Fig (٢-١)



(٢-٢-٥-٢) Prepartion of Reagent

١- The precipitating solution: - Tri chloroacetic acid (TCA) ٥٠٪ (٥٠gm of TCA are dissolved in a final volume of ١٠٠ ml of DDW).

٢-Ethylene diamine tetra acetic acid-di sodium (EDTA-$Na_٢$)(٠.٤M) (١٤٨.٩gm of EDTA are dissolved in a final volume of ١ litter of DDW).

٣-Tris-EDTA buffer (٠.٤) (pH = ٨.٩)

٤٨.٤٥٨ gm of tris is dissolved in ٨٠٠ ml of DDW. ١٠٠ ml of (٠.٤M) EDTA solution are added and brought to a final volume of ١ liter with DDW. The pH is adjusted to ٨.٩ by the addition a drops of ١M of HCl.

٤- DTNB reagent (٠.٠١M)

٠.٠٩٩ gm of DTNB is dissolved in absolute methanol and brought to a final volume of ٢٥ ml (this reagent stablize for at least ١٣ week at ٤ ^{o}C).

٥- GSH standard

Stock standard solution (٠.٠٠١M) is prepared by dissolving ٠.٠٣٠٧ gm of a GSH in a final volume of ١٠٠ ml of (٠.٤M) EDTA **solution.** Dilution is made in EDTA solution to ٢,٥,١٠,٢٠,٣٠ and ٤٠µM. (This working standard solution should be prepared daily).

(٣-٥-٢-٢) Procedure

Serum GSH was determined by using a modified procedure utilizing Ellman's reagent (DTNB), which can be summarized as follows.

Duplicates of each standard and sample test tubes are prepared, then piped into test tubes.

Reagents	**Sample µl**	**Reagent black µ l**	**Standard µ l**
Serum	١٠٠	-----	-----
Standard	-----	----	١٠٠
DDW	٨٠٠	٩٠٠	٨٠٠
TCA	١٠٠	١٠٠	١٠٠

Tubes are mixed in vortex mixture intermittently for ١٠-١٥ minutes, and

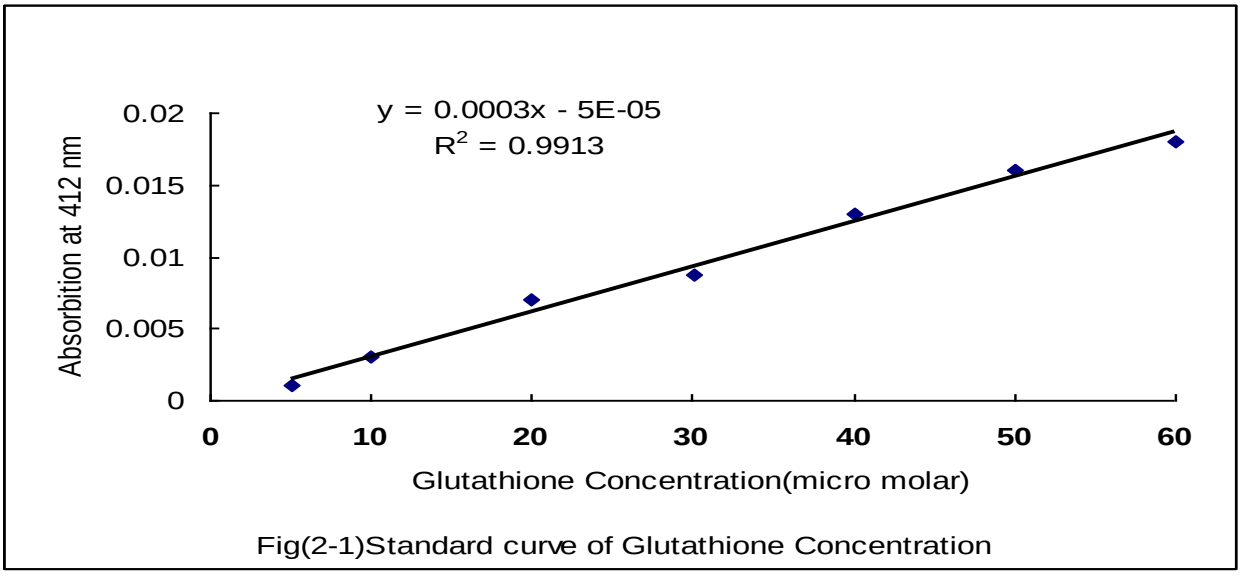
Centrifuge for ١٥ minutes at ٣٠٠ xg, then piped into test tubes.

Reagents	Sample µ L	Reagent blank µL	Standard µL
Supernatant	٤٠٠	٤٠٠	٤٠٠
Tris EDTA buffer	٨٠٠	٨٠٠	٨٠٠
DTNB reagent	٢٠	٢٠	٢٠

Tubes are mixed in vortex mixture. The spectrophotometer adjusted with reagent blank to read zero absorbance at ٤١٢ nm, and the absorbance of standards and sample is read within five minutes of the addition of DTNB.

(٢-٢-٥-٤): -Calculation of Serum GSH

The concentration of GSH is obtained from the calibration curve in µM (fig-٢-٢).



Fig(2-1)Standard curve of Glutathione Concentration

(٢-٢-٦) Determination of Serum Uric Acid [١٥٤,١٥٥]

The determination of uric acid in serum was measured enzymatically by the biomagreb kit.

(٢-٢-٦-١) Principle

Uric acid is oxidized to allantoin and hydrogen peroxide by the uricase, according to the following equation: -

$$Uric\quad acid + O_2 + H_2O \xrightarrow{Uricase} Allantoin + CO_2 + H_2O_2$$

$$H_2O_2 + 3{,}5\,dichloro-2-hydroxybe\,n\,z\,ene\ sulfonic\,acid + 4-a\min o\,antipyrine \xrightarrow{peroxidase} Chromogen + HCl + 2H_2o$$

(٢-٢-٦-٢) Reagents

The reagant used in this test is a mixture of: -

١- tris buffer pH ٨.٠ (٥٠ mmol/l)

٢- ٣.٥ dichloro-٢-hydroxybenzene sulfunic acid ٢ mmol/l.

٣- surface-active agent ٢ mmol/l.

٤- uricase ≥ ١٠٠ u/l.

٦- ascrobate oxidase ≥ ١٠٠٠ u/l.

٧-aminoantipyrine ٠.٢٥ mmol/l.

٢.٢.٦.٣ Procedure

	Reagent blank	**Standard**	**Sample**
Standard (٨mg/dl)	-----	**٢٠µl**	-----
Sample	------	------	**٢٠µl**
Working solution	**١ ml**	**١ ml**	**١ ml**

After addition, mix the content of every tube, allow to stay at room temperature for ١٠ min or incubate for ٥ minute at $37^{\circ}C$ and read absorbance value spectrophotomerically at wave length ٥٢٠ nm, beyond to the formation of the quinoneminewhich dye is proportional to the uric acid concentration in serum.

Uric acid (μMol/l) = (A (absorbance of sample/A absorbance of standard) X ٣٥٧

(٢-٢-٧) Determination of Serum Total Cholesterol [١٥٦]

Total cholesterol in the serum was measured by enzymatic method, with the biomerux kit, France.

(٢-٢-٧-١) Principle

The principle of this method was lysis of the cholesterol ester to produce cholesterol and fatty acids, then oxidized to produce the quinoemine: -

$$\text{Cholesterol ester} \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol} + \text{fatty acid.}$$

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

$$2H_2O_2 + Phenol + 4-a\min o\ antipyrine \xrightarrow{peroxidase} quino\,e\min e + 4H_2O$$

(٢-٢-٧-٢) Reagents

The reagent used in test is a mixture of: -

١- phosphate buffer ٠.١ mol/l.

٢- phenol ١٥ mmol/l.

٣- sodium cholate surfactante ٣.٧٤ mmol/l.

٤- ٤ amino antipyrine ٠.٥ mmol/l.

٥- peroxidase ≥ ١٠٠٠ U/l.

٦- Cholesterol oxidase ≥ ٢٠٠ U/l.

٧- Cholesterol esterase ≥ ١٢٥ U/l.

(٢-٢-٧-٣) Procedure

The procedure for this method is as follows: -

	Reagent blank	Standard	Sample
Standard ٢٠٠ mg/dl	----	١٠µL	
Sample			١٠µL
Working reagent	١ ml	١ ml	١ ml

After addition, mix the content of every tube.

Allow staying at room temperature for ١٠ minutes or incubating at $37^{\circ}C$ for ٥ minutes and reading absorbance by spectrophotometer at ٥٠٠ nm. The intensity of the produced color is directly proportional to the total cholesterol concentration in the sample

$$Total\ cholesterol\ (mmol/l) = \frac{A_{bs}\ of\ sample}{A_{bs}\ of\ standard} X\ 5.17$$

(٢-٢-٨) Determination of Serum Triglyceride[١١١]:-

Total triglycerides in the serum were measured by enzymatic with the (biomerieux kit, France).

(٢-٢-٨-١) Principle

Total triglyceride determination depends on formation of quinonemine by using a group of enzymes as fellows: -

$$Triglyceride \xrightarrow{lipase} Glycerol + fatty\ acids$$

$$Glycerol + ATP \xrightarrow{Glycerol\ kinase} Glycerol - 3 - phate + ADP$$

$$Glycerol - 3 - phosphate \xrightarrow{G-3-\vec{p}\ oxidase} Dihydroxy\ acetone\ phosphate + H_2O$$

$$2H_2O_2 + Pchlorophenol + 4 - a\min o\ antipyrine \xrightarrow{peroxidase} Quinone\min e + 4H_2O$$

(٢-٢-٨-٢) Reagents

The reagent used in this test is a mixture of: -

١- tris buffer PH ٧.٦	١٠٠ mmol/l.
٢- p-chlorophenol	٢.٧ mmol/l.
٣- Magnesium	٤ mmol/l.
٤- ٤ aminoantipyrine	٠.٤ mmol/l.
٥- lipase	≥ ١٠٠٠ U/l.
٦- Glcerol kinase	≥ ٢٠٠ U/l.
٧- Glycerol-٣-phosphate oxidase	≥ ٢٠٠٠ U/l.
٨- Peroxidase	≥ ٢٠٠ U/l.
٩- ATP	٠.٨ mmol/l.

(٢-٢-٨-٣) Procedure

	Reagent blank	Standard	Sample
Standard ٢٠٠ mg/dl	----	١٠µl	-----
Sample	-----	------	١٠ µl
Working reagent	١ ml	١ ml	١ ml

Gently mix the content of every tube after addition, allow staying at room temperature for ١٠ minute or incubating at ٣٧° for ٥ minute and reading spectrophotometrically at ٥٠٥ nm. The intensity of the produced color is proportional to the total triglyceride in the sample.

$$\text{Total serum triglyceride (mmol/l)} = \frac{Abs.of\ sample}{Abs.of\ s\tan dard}\ X\ 2.26$$

(٢-٢-٩) Determination of Serum High Density Lipoprotein-Cholestrol (HDL-C)[١١٢]

HDL-Cholesterol in the serum were measured by enzymatic method using biomeriex kit, France.

(٢-٢-٩-١) Principle

The addition of the phosphotungestic in the presence of magnesium ions is due to precipitate the chylomicrons and lipoproteins of very low density (VLDL) and low density (LDL) contained in the sample.

The supernatant obtained after centrifugation contains high-density lipoprotein fraction HDL measured by enzymatic method.

(٢-٢-٩-٢) Reagents

Reagent(١) Precipitation Reagent	**Phosphotingestic acid MgCl٢.٦HO pH ٦.٢**	**٤٠ g/l ١٠٠ g/l**
Reagent (٢) HDL ch. Calibrating solution	Free esterified Cholesterol	١.٣٠mmol/l ٠.٥ g/l

(٢-٢-٩-٣) Procedure

	Reagent blank	**Standard**	**Sample**
Distilled water	٥٠ µl	-----	------
HDL-calibrator	-----	**٥٠µL**	-----
Supernatant	----	-----	**٥٠µL**
Working reagent	١ ml	١ ml	١ ml

The working solution is the cholesterol enzymatic solution. Gently mix the content of every tube after addition, allow staying at room temperature

for ١٠ minutes or incubating for ٥ minutes at $37^{\circ}C$ and read spectrophotometrically at ٥٠٠ nm.

The concentration of HDL- cholesterol

$$HDL-(mmol/l)=\frac{Abs.of\ sample}{Abs.of\ s\tan dard}\ X\ 1.42$$

١.٤٢(the concentration of standard).

(٢-٢-١٠) Determination of Serum Low Density Lipoprotein-Cholesterol LDL: -

In lipoprotein fractionation the widely accepted method for determining the LDL-cholesterol is the beta quantification procedure. Very low-density lipoprotein (vLDL) is separated by ultra centrifugation and high-density lipoprotein (HDL), by precipitation. Because of ultra centrifugation is unavailable in most routine laboratories and the procedure is expensive, time consuming and technically demanding, the nearly universal approach in clinical laboratories has been to estimate LDL-cholesterol from the formula of (Friedewaid *et al*.١٩٧٢)[١٦٣,١٦٤]. After the measurement of total cholesterol, triglycerides and HDL-cholesterol, LDL-cholesterol is calculated from the equation:-

LDL= TC - VLDL (estimated as triglyceride ÷٢.٢) - HDL [١٦٣,١٦٤]

(٢-٢-١١) Determination of Serum Lipid Peroxidation: -

The concentration of lipid peroxides in the plasma was determined by the colorimetry thiobarbituric acid (TBA) method.Under the acid and heating conditions of the reaction the lipid peroxides break down to form malondialdehyde (MDA) which is complex with (TBA). The resulting MDA-TBA chromogen can be measured spectrophotometrically at ٥٣٢[١٦٥,١٦٦].

(٢-٢-١٠-١) Reagents

١- ٠.٦ (W/V)% TBA (٠.٦ gm of thiobarbituric acid in ١٠٠ ml D.W).

٢- ١٧.٥ (W/V)% (١٧.٥ gm of trichloroacetic acid in ١٠٠ DW)

٣- ٧٠(W/V)% (٧٠ gm of trichloroacetic acid in ١٠٠ DW).

(٢-٢-١٠-٢) Procedure

١- ٢٥٠ µl of serum was added to the test tube.

٢- ١ ml of ١٧.٥٪ TCA was added to the tube of sample.

٣- Place the tube in ice then add ١ml of ٠.٦٪ TBA.

٤- Place in boiling water baths for ١٥ minutes and then allow it to cool.

٥- Add ١ml ٧٠٪ TCA then to incubate for ٢٠ minutes.

٦- The sample was then centrifuged for ١٥ min at ٢٠٠٠ rpm then measure the absorbance spectrophotometrically of the supernatant at the wave length ٥٣٢ nm against a reagent blank. The preparation of reagent blank was the same procedure above except changing the sample with DW.

Serum MDA= (Absorbance/d* ε) / D.F [١١٥]

d = ١cm, ε =extinction coefficient = ١.٥٦x ١٠^{٥} M$^{-١}$cm$^{-١}$

D.F = dilution factor = ٤

(٢-٣)Statistical analysis

The results are expressed as number, range, confidence interval C.I ٩٥٪ and whenever possible as mean ∓ SD (SE) of number of observations. The data are analyzed by using student's "t" and correlation test taking P≤٠.٠٥ as the lowest limit of significance.

CHAPTER THREE

٣- Results and Discussion: -

In the present study, the control values of different variants were determined in sera of apparently healthy individuals, compared with different in-patients group. Then, the determination of different variants was done in sera of patients with the two types of DM.

The age of healthy controls and patients subjected to the present study are shown in Table (٣-١). Groups, sex, No., mean (years), SD (upper value, lower value).

Table (٣-١) The age of healthy controls and patients with diabetes mellitus.

	No.	Sex	Mean	SD	Upper	Lower
Control	٣١	Male	٣٥.٢	٩.٢٢	٦٠	٢٢
Control	١٩	Female	٣٢.٦	٨.٧	٥٧	٢٢
Type ١	٢١	Male	٣٠.٨	١٠.٧	٥٥	٢٨
Type ١	٩	Female	٣١.٠٧	٩.٣	٥٦	٢٧
Type ٢	١٥	Male	٣٨.٧	١٠.٢	٦٢	٣٦
Type ٢	٩	Female	٣٧.١١	١٠	٥٥	٣٨

٣-١ The Effect of Diabetes on Creatine Kinase: -

Compared with healthy controls, CK activity was found to be significantly decreased in sera of patients with diabetes mellitus types Iand II as shown in Table (٣-٢) and Fig (٣-١).

Table (٣-٢) CK activity (U/L) in sera of patients and healthy controls.

M=males, F= females, S= significant (when P<٠.٠٥), NS= not significant).

	Sex	Mean	SD	CK		SE	٩٥٪ C.I.		P	Sign
				Upper	Lower		Upper	lower		
Control	M	١١٥.٥٨	٢٥.٢	١٨٠	٧٦	٤.٢٥	١٢٢.٨	١٠٥.٦٣	--	--
	F	١١٢.٨	٣٨.٧	١٨٣	٧٠	٨.٨٧	١٣٥.٩٥	٩٥.٨٢	--	--
Type ١	M	٨٦.٨	٣٦.٨	١٤٠	٥٦.٦	٧.١٤	١٠٦.٥٨	٧٠.١٨	٠.٠٠١	Sign
	F	٨١.٢٣	٣٤.٩٨	٩٨.٤	٥٢.٨	١١.٢٦	١٠٦.٧	٥٥.٧٦	٠.٠٠١	Sign
Type ٢	M	٨٧.٣٣	٣٣٣١	١٦٨	٥٠	٨.٦	١٠٦.٧٨	١٠٠.٣٣	٠.٠٠١	sign
	F	٧٩.٥٥	٣٨.٣٧	١٥٠	٥٠	١٢.٧٩	١٠٨.٤٨	٥٠.١٦	٠.٠٠١	sign

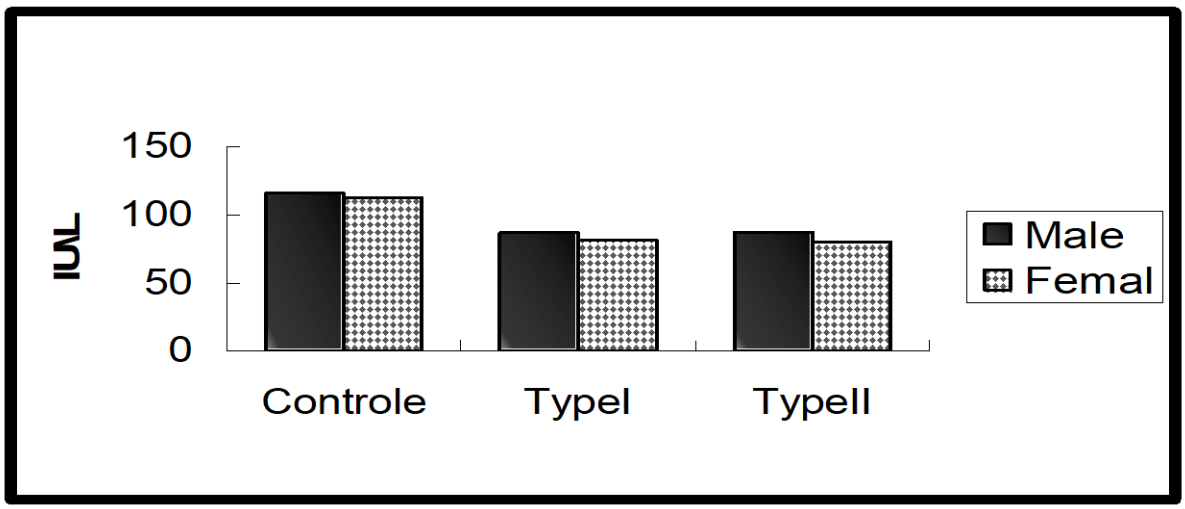


Fig (٣-١) The level CK activity in sera of patients and healthy controls.

There is much evidence, which suggests that, the decrease of CK activity in serum is beyond ROS: -

١- Oxygen free radicals are implicated in mediating various pathological processes such as diabetes [١٦٢,١٦٩]. Free radicals are known to interact with enzyme and other biomolecules and affect their structure and function then leads pathophysiological conditions. The active sites of CK contain cysteine residues, which are very essential for enzyme activity and substrate binding [١٧٠]. This cysteine could be the targets for oxygen free radicals due to the modification of this group could be the reason for the enzyme inactivation. The inactivation effect of super oxide radical and hydroxyl radical was reversible while the inactivation effect of peroxinitrite was reported to be irreversible [٨٩,٩٠,١٧١].

The addition of SOD or CAT almost completely reveres the effect of superoxide or H_2O_2 on the CK activity respectively [١٦٩]. The depressed activities of CK due to oxygen free radicals were reversed to control values by DTT.

This protection can be due to two reasons; the first is that this compound reduces the oxidized sulfhydryl group of enzyme at the active site oxidized by oxygen free radicals or the sulfhydryl group of DTT may react directly with oxygen free radicals protecting the enzyme active sites from the attack by oxygen free radicals.

Free radicals of x-ray-induced water radiolysis via their reaction products reduce the activity of CK [٥٦].

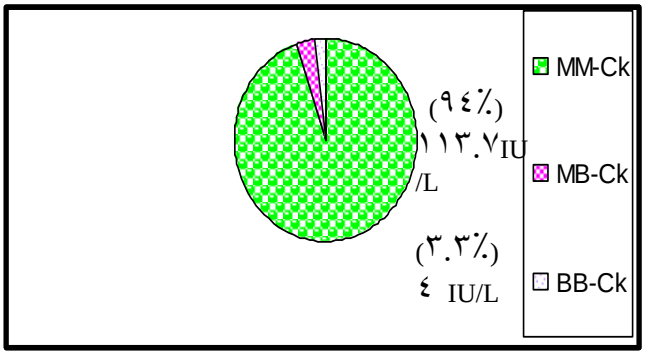
٢- Alloxan and streptozotocin are widely used to induce experimental diabetes in animals. The mechanism of their action in ß-cells of the pancreas has been found to occur by the action of nitric oxide [١٧٢]. Hoeldtke *et al* [١٧٣]. And Chiarelli *et a*l [١٧٤] demonstrated *in vivo* that the nitrosative stress increases in DM. Increasing Nitric oxide is due to inhibition creatine kinase [١٧٥]. $\dot{N}D$ Reduces the activity of creatine kinase irreversibly via nitrosylation of the sulfur centers [١٧٦] (creatine kinase possesses eight sulphydryl group)[١٧٧].

٣- MM-CK incubated with (hypo) xanthine plus xanthine oxidase inhibited to lower value of activity, adding DTT or GSH prevents this effect [٨٠].

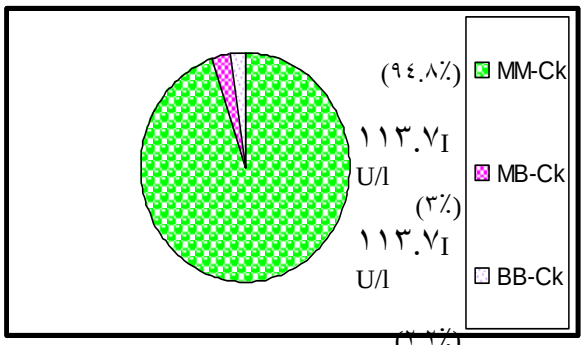
٤- Hassan Mekhfi *etal* demonstrated that creatine kinase is the main target of reactive oxygen species in cardiac myofibrils [١٧٨].

All these observation suggest that decreased creatine kinase activity occurs because of the effect of free radicals on the thiol group (a constant amount of protein but decrease the CK activity) .The decrease of creatine kinase may occur also because of diabetes decreased CK-Mm-RNA) to ٦١.١٪ [١٧٩]due to decreased CK-M sub unit. (decrease the amount of protein).

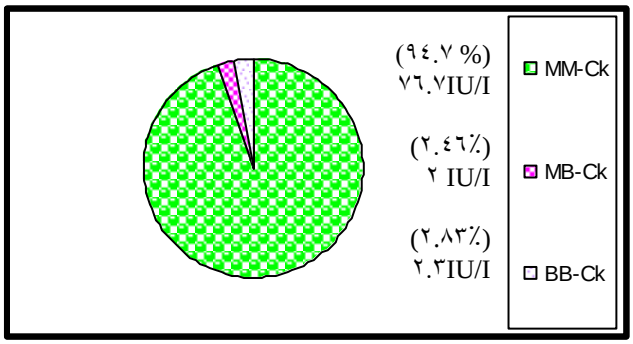
The result of this study is more agreement with the last suggestion because (BB-CK) stays constant relatively as shown in Fig (٣-٢).



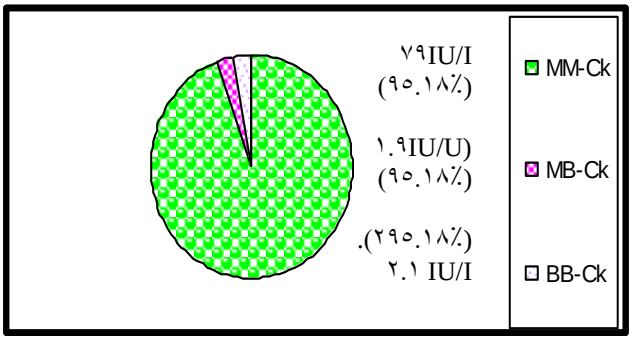
The levels of CK isoenzymes in control (male)



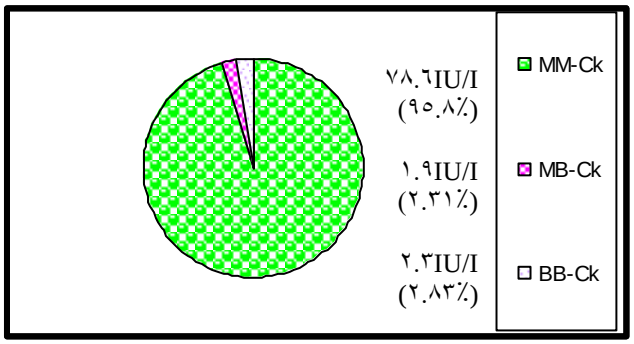
The levels of CK isoenzymes in control (female)



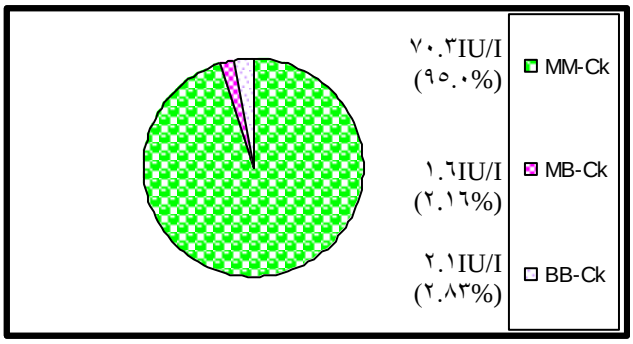
The levels of CK isoenzymes in Type I (male)



The Levels of CK isoenzymes in Type I (female)



The levels of Ck isoenzymes in Type II (male)



The levels of Ck isoenzymes in Type II (female)

Fig (٣-٢) The Levels of CK Isoenzymes(IU/L) in Controls and Patients with Diabetes Mellitus Type I and Type II

٣-٢ The Effect of Diabetes on Glutathione: -

Glutathione (GSH), cysteine and ergothionine are three compounds, which make up the non-protein thiol compounds (NPSH)[١٨٠]. Many investigators using methods, which measure NPSH, have interpreted the result as GSH because GSH makes at least ٩٠٪ of the NPSH [١٨١]. GSH plays a central role in the defense against a variety of diseases. Its function includes the detoxification of carcinogens, free radicals and peroxides, regulation of immune function; and maintenance of protein structure, function and turnover [١٨٢].

Compared with healthy controls, GSH concentrations were found to be significantly decreasing in sera of patients with different types of Diabetes mellitus. In the present study it was found that GSH is decreased statistically significantly as shown in table (٣-٣) and fig (٣-٣). Previous studies have shown low levels of GSH in patients with renal failure, liver failure [١٨٣], and CHD [١٨٤] vitamin B١٢ deficiency [١٨٥] and multiple-organ failure [١٨٧].

Table (٣-٣): -The levels of GSH in healthy controls and patients with diabetes mellitus.

	Sex	Mean	SD	GSH(µmol/l)		SE	٩٥٪ C.I		P	Sign
				Upper	Lower		Upper	Lower		
Control	M	٣٠.٢٢	٥.٠٦	٣٢.٣	١٧.٣	٧.٠٥	٤٦.١٦	١٤.٢٧	--	--
	F	١٩.٣٩	٥.٠٤	٢٩.٣	١٤.٣	١.١٥	٢٢.٠	١٦.٧	--	--
Type ١	M	١٧.٤٦	٥.٦٣	٢٧.٦	١١.٦	١.٠١	١٩.٩٥	١٥.١٧	٠.٠٠١	Sign
	F	١٦.٦٧	٥.٣٨	٢٤.٣	٧	١.٧٩	٢٠.٧١	١٢.٣٧	٠.٠٠١	Sign
Type ٢	M	١٦.٦٥	٢.٤١	٢١.٠	١٣.٤	٠.٦٢	١٨.٠٦	١٥.٢٣	٠.٠٠١	Sign
	F	١٢.٦١	٤.٧٣	٢١.٠	٧	١.٥٧٧	١٧.١٧	١١.٣	٠.٠٠١	Sign

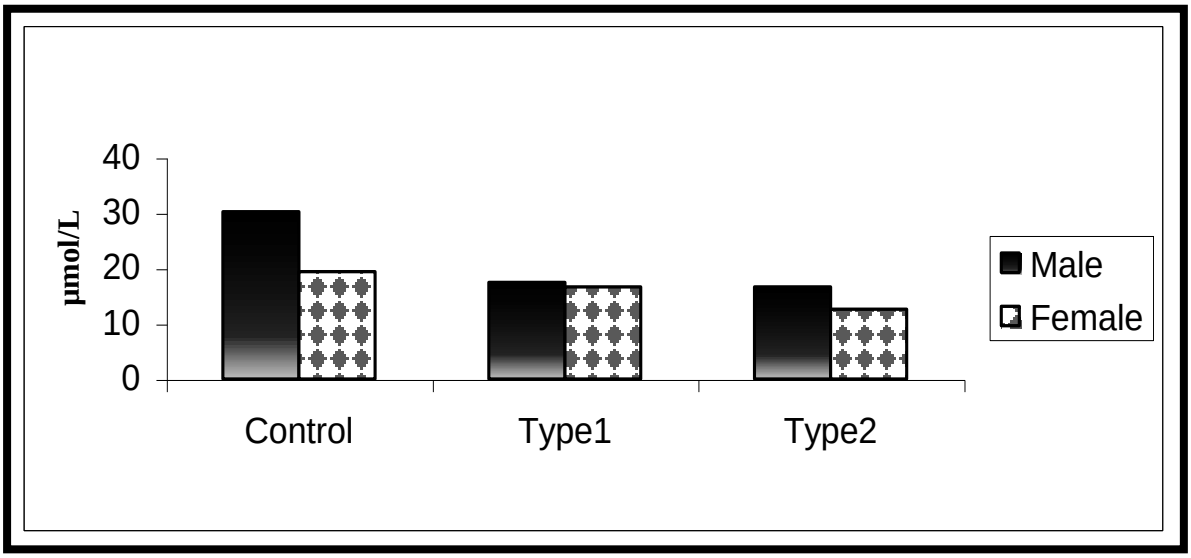


Fig (٣-٣) **The levels of GSH in healthy controls and patients with diabetes mellitus.**

The decrease of GSH in diabetes may be attributed to

(١) Decreasing the synthesis of GSH: by using C^{14}-glycine, the synthesis of GSH by liver slices from the diabetic rat is found to be below the normal rate [١٨٦].

(٢) Increasing the rate of producing free radicals in diabetes mellitus; in this case GSH acts as scavenger and that is due to the decrease in GSH. (Alloxan destroys B-cell via producing free radicals; a single dose of GSH inhibited the function of alloxan) [١٨٠].

Intracellular GSH has a protective role against oxidative damage by neutralizing vitamin E or by the action of GSH peroxidase; it catalyzes the reaction: -

$$H_2O_2 + 2GSH \rightarrow GSSG + 2H_2O$$

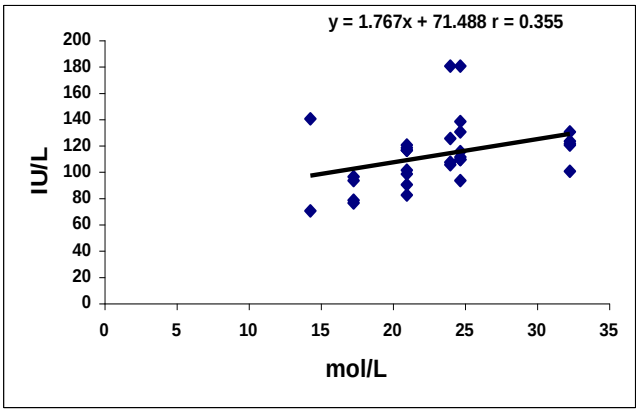
There is a family of GSH peroxidase rather than one enzyme all members of which contain subunits, which has one selenium atom at the active site [١٨٨].

Eldjarn *et al* demonstrated that SS/SH equilibiria are more favored than finding different concentrations from one of their [١٨٩]. This fact adds on a second possible reaction between GSH and protein disulphide group to produce a protein- non-protein disulphide compound: -

$$GSH + protein - S - S - protein \rightarrow protein - S - H + G.S.S - protein$$

The depletion of GSH levels in the present study is compared with healthy controls supports the hypothesis that considers GSH as a protective factor against the development of different types of Diabetes mellitus.

Fig (٣-٤): - shows the different correlations between CK activity and glutathione in control, type I and type II diabetes mellitus. A previous study has shown this correlation to indicate multiple-organ failure [١٧٩].



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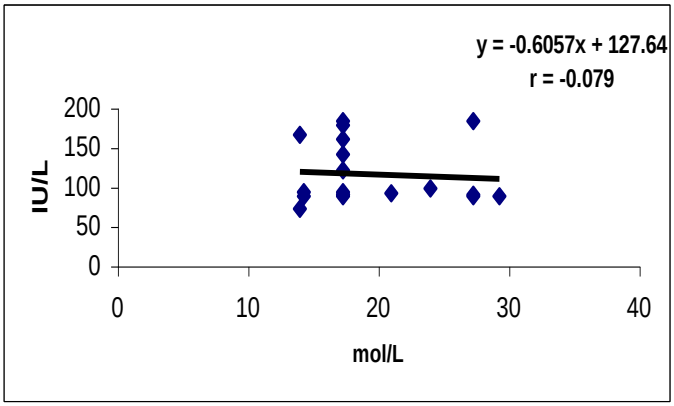

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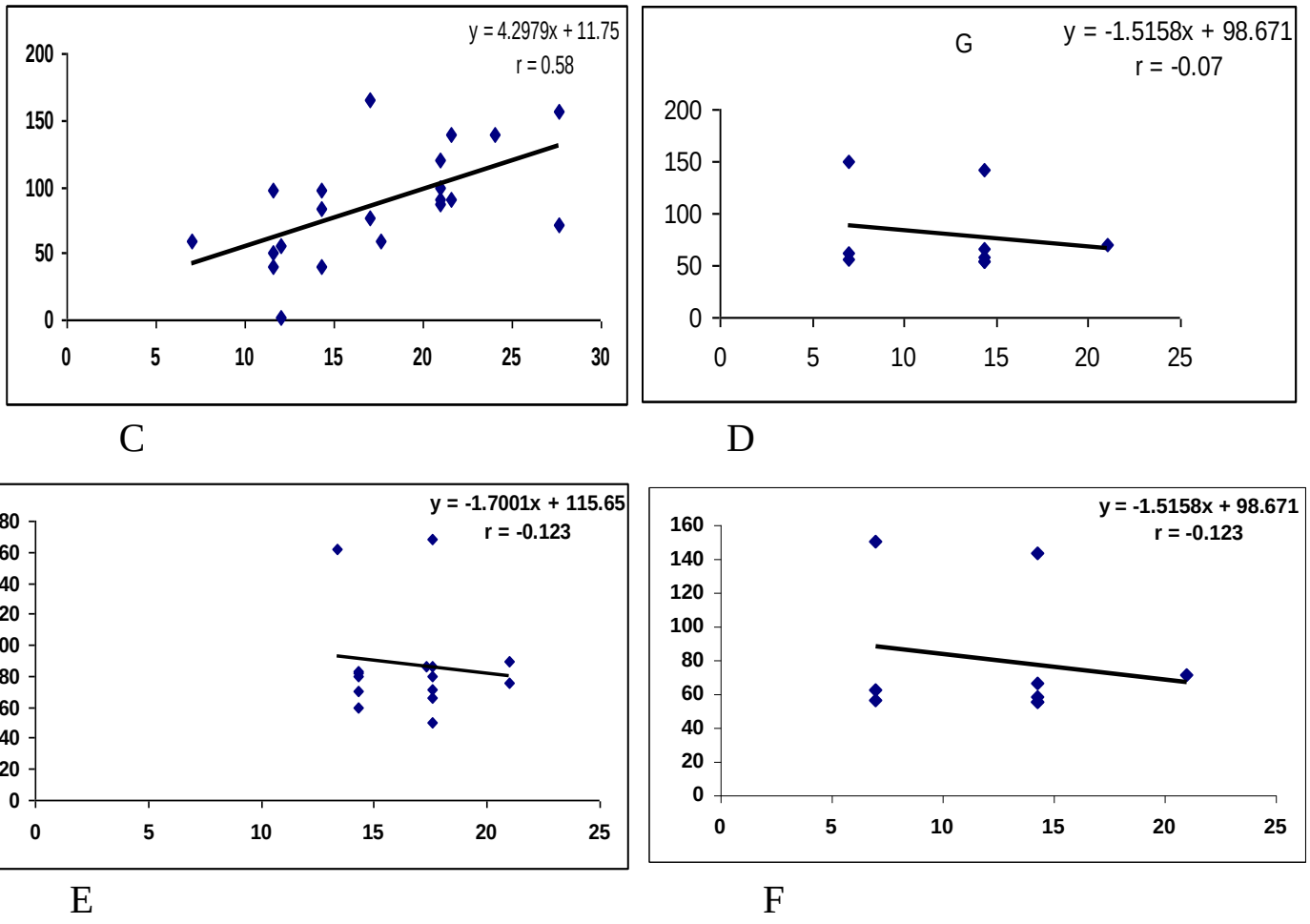


Fig (٣-٤) The different correlations between CK activity and glutathione.

A (Control male), B (Control female), C (Type I male), D (Type I female), E (Type II male), F (Type II female)

٣-٣ The Effect of Diabetes on Uric Acid: -

Uric acid is generated in the human body by the oxidation of purines. No enzymes are required to oxidize it further. It possesses free radical scavenging properties and systemic administration is known to increase serum antioxidant capacity [١٩٢]. A previous study [١٩٠] found a

significant inverse relationship between serum UA concentration and oxidative stress during acute aerobic exercise. UA reacts with oxygen-derived free radicals and becomes oxidized in skeletal muscles [١٩١].

Compared with healthy controls, UA concentrations were found to significantly decrease in sera of patients with different types of Diabetes Mellitus, In the present study its found that UA decreases statistically significantly as show in Table (٣-٤) and Fig (٣-٥).

Table (٣-٤): -The levels of UA in sera of healthy controls and patients with diabetes mellitus.

	Sex	Mean	SD	UA(µmol/l)		SE	٩٥٪ C.I		P	Sign
				Upper	lower		Upper	Lower		
Control	M	٢٢٥.٦	٢٧.٨	٢٨٠	١٩٢	٥.٥٦	٢٣٨.١٨	٢١٣.٠٣	--	--
	F	٢١٤.٦	١٩.٣	٢٨٠	١٩٣	٧.٢	٢٢١.٨٣	١٩٨.٣٣	--	--
Type I	M	١٩١.٩	١٣.٣	٢٤١	١٨٠	٢.٤	١٩٧.٥٨	١٨٦.٣٢	٠.٠٠١	Sign
	F	١٨١.١	١٧.١٦	٢١٠	١٨٠	٥.٧٢	١٧.٤٧	١٦٨.٨	٠.٠٠١	Sign
Type II	M	١٧٨	١٠.٤	٢٠٠	١٧٠	١٥.١٢٣	٢١٢.٢	١٤٣.٧٩	٠.٠٠١	Sign
	F	١٨٠.٧	١٤.٤٣	١٩٨	١٥٠	٤.٧٩	١٩١.٦	١٧٧.٩٣	٠.٠٠١	sign

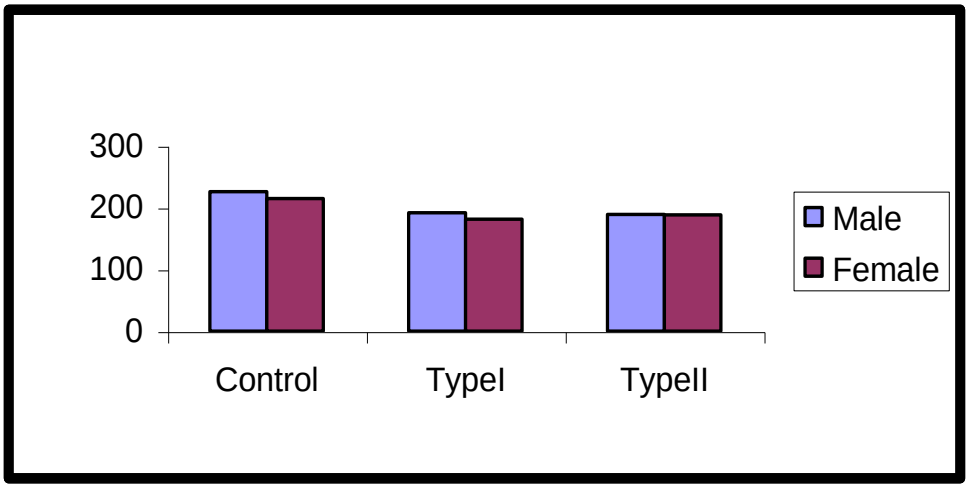


Fig (٣-٥) The levels of UA in healthy controls and patents with diabetes mellitus.

A previous study has shown low levels of UA in patients using the drug "pyrazinamide" because this drug induces increment urate excretion [١٩١]. Increased dietary protein elevates plasma uric acid and is associated with decreasing oxidative stress [١٩٣]. Increasing UA is associated with stroke [١٩٤,١٩٥].

The decrease of UA in diabetes may be related to

١-UA acts as scavenger for peroxynitrate [١٩٦] ((this radical increases in DM· [١٧٢,١٧٤])) to produce nitrated uric acid.

Fig (٣-٦)[١٩٦].

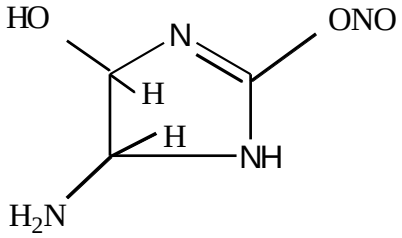


Fig (٣-٦) Proposed structure of nitrated uric acid product [١٩٦]

٢- Nitrogen dioxide depletes uric acid [١٩٧], this compound increases in DM [١٧٢,١٧٤].

٣- Chiarelli *et al* [١٧٤] suggest a mechanism which occurs because of increasing nitric oxide and nitrogen peroxy nitrate. This mechanism produces hyper filtration,which may be due to the decrease in UA in serum and the increase in urine . Fig (٣-٧).

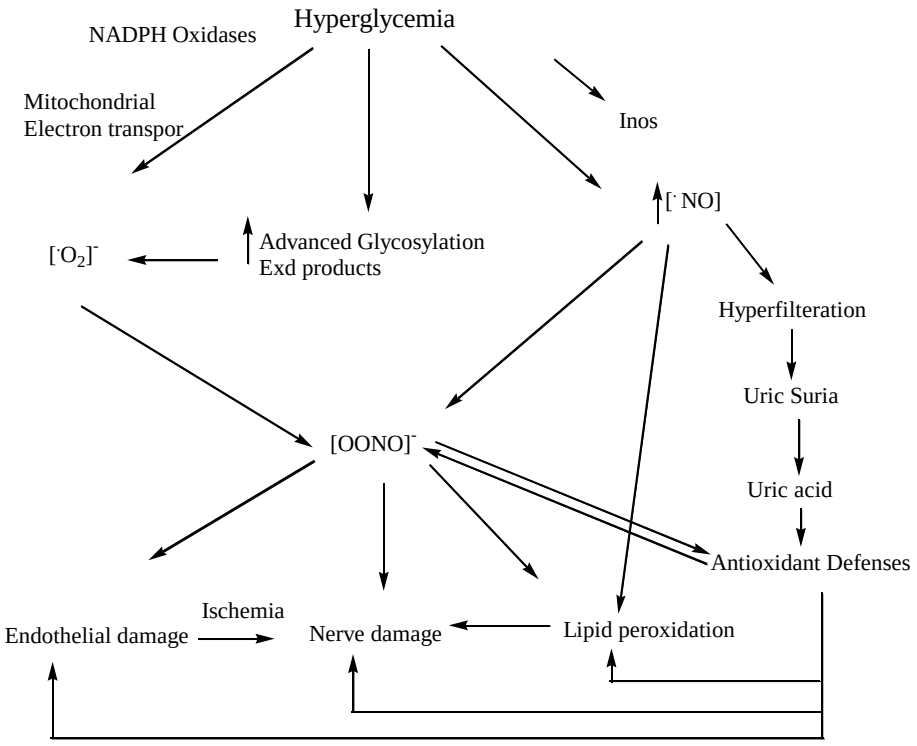
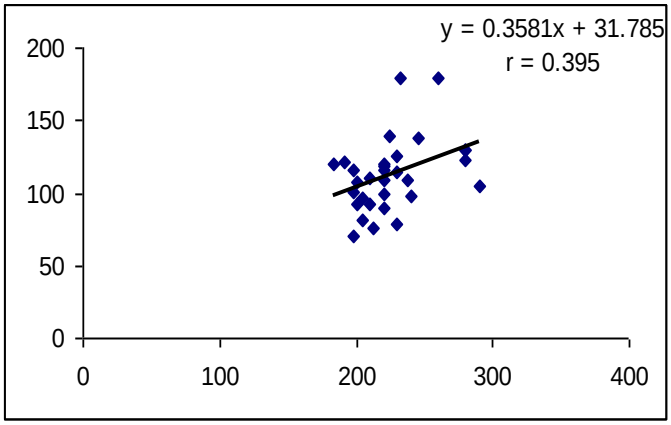


Fig (٣-٧) Nitrosative stress and hyperfiltration "cited from [١٧٣]„

Fig (٣-٨) shows the different correlation between CK activity and UA in control, type one and type two. A previous study has shown this correlation to indicate multiple-organ failure [١٧٩].



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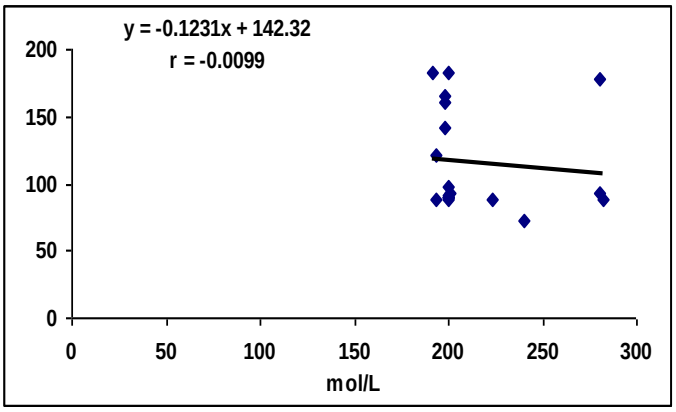
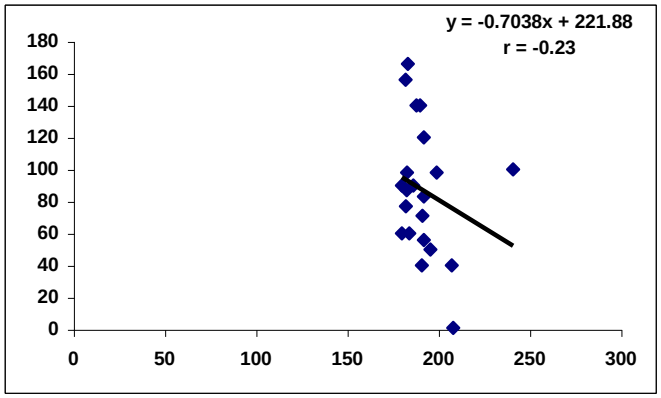
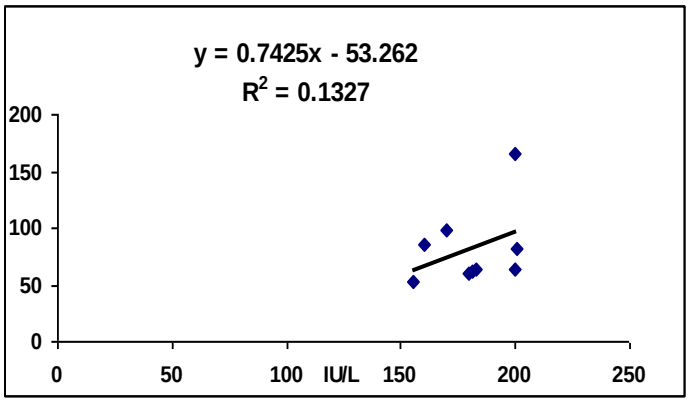


B



B



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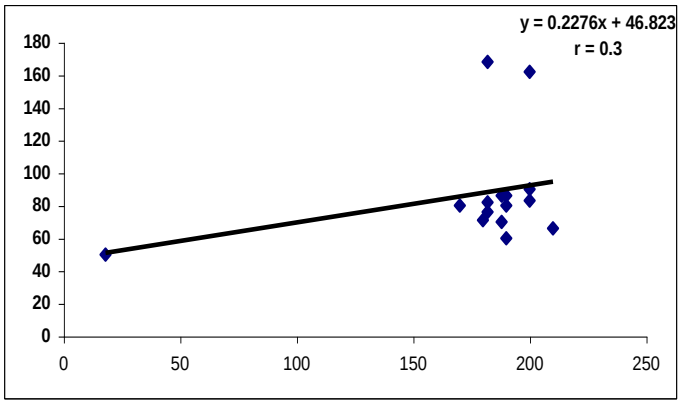

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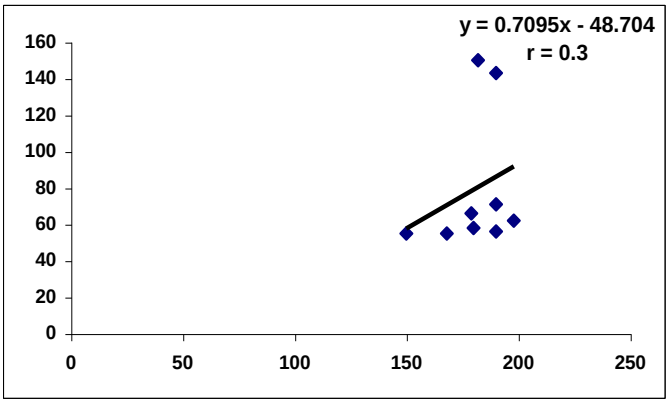


E

Fig (٣-٨) The Different correlations between CK activity and UA, A (control male), B (Control female), C (Type I male), D (Type I female), E (Type II male), F (Type II female)

(٣-٤) The Effect of Diabetes on Total Cholesterol

Unsaturated fatty acid in phospholipids and also cholesterol in cell membranes act as a susceptible to free radical and singlet oxygen $(^1O_2)$-mediated peroxidative modification, during which lipid hydroperoxide (LOOH) derivatives may accumulate [١٩٩,١٩٨].

There are many causes which make the LOOHs noxious to a cell; these reasons include (١) relatively high polarity, which can perturb membrane structure (٢) long life time compared with free radical precursors or products [٢٠٠], (٣) involvement in oxidative stress signaling that could culminate in a peptic cell death [٢٠١].Compared with healthy controls. Total cholesterol concentrations were found to significantly increase in sera of patients with type I and type II Diabetes mellitus as shown in Table (٣-٥) and Fig (٣-٩).

Previous studies have shown that the role of diabetes on cholesterol synthesis is unclear [٢٠٢,٢٠٣,٢٠٤]. The effects on cholesterol metabolism of normalizing blood glucose levels with insulin have varied from decrease [٢٠٥] to unchanged [٢٠٢]. The increment of total cholesterol in the present study, when compared with healthy control, supports the hypothesis that considers glucose increasing is due to increase in cholesterol synthesis.

Table (٣-٥) Total cholesterol levels in healthy controls and patients with diabetes mellitus.

	Sex	Mean	SD	TC(mmol/l)		SE	٩٥٪ C.I		P	Sign
				Upper	lower		Upper	Lower		
Control	M	٤.٥٩٦	٠.٣٠٩	٤.٨	٣.٨	٠.٠٧٢	٦.٩٣	٣.٠٤	--	--
	F	٤.٣٠٧	٠.٥٩٦	٥.٠	٣.٩	٠.١٤٥	٤.٦٣٥	٣.٩٧	--	--
Type I	M	٤.٩	٠.٩٦	٥.٦٩	٣.٩٨	٠.١٧٣٨	٥.٣٤	٤.٥٥	٠.٠٥	Sign
	F	٤.٤٦	٠.٧٤	٤.٩٤	٣.٤	٠٢٤	٥.٠٢٥	٣.٨٩	٠.٠٥	Sign
Type T	M	٤.٧٠٦	٠.٩٦	٦.٢	٣.٩٢	٠٢٤٨	٥.٢٦	٤.١٤٥	٠.٠٥	Sign
	F	٤.٤٦	٠.٨٧	٦.٦	٣.٦	٠.٢٩	٥.١١	٤.٤٦	٠.٠٥	sign

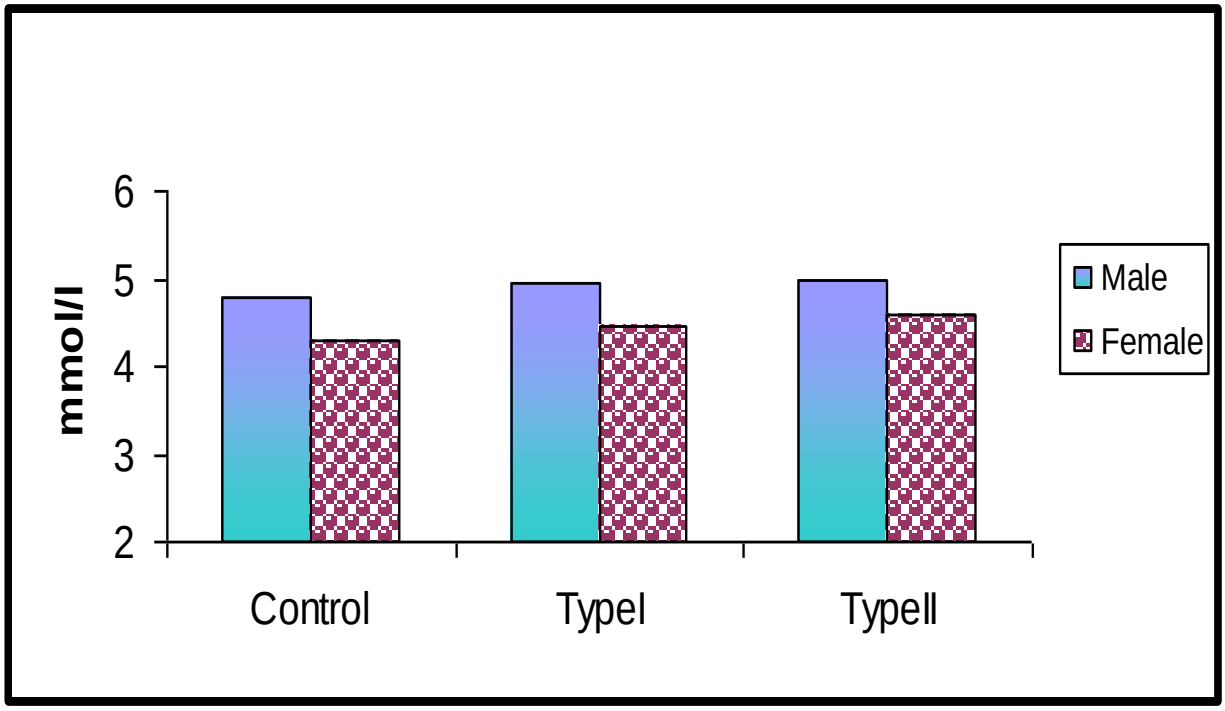
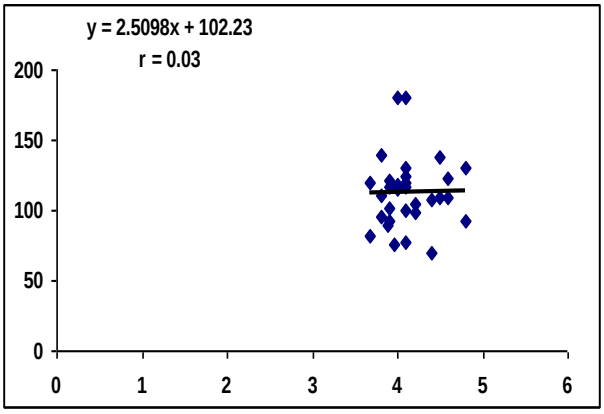
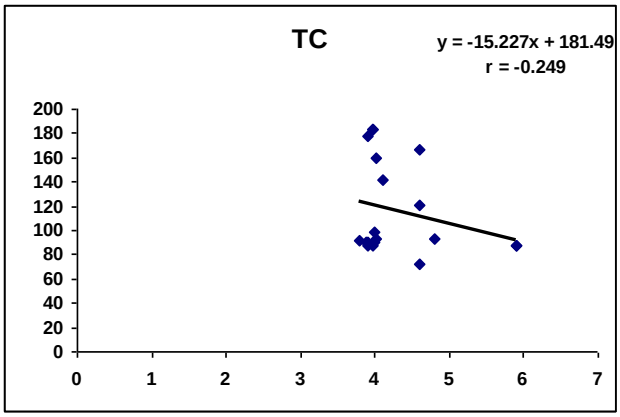


Fig (٣-٩) The levels of TC in sera of healthy controls and patients with diabetes mellitus

The different correlations between TC.and CK activity in healthy controls and patients with diabetes mellitus are shown in Fig (٣-١٠)



A



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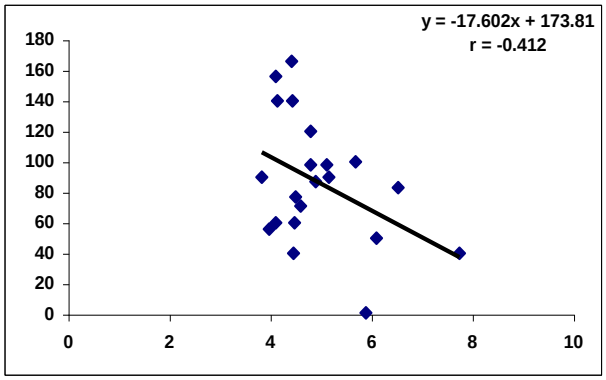

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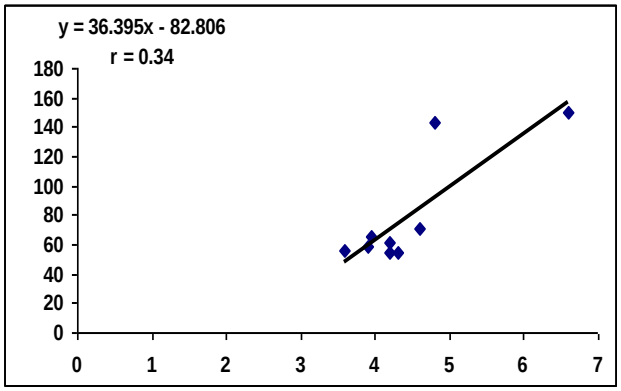

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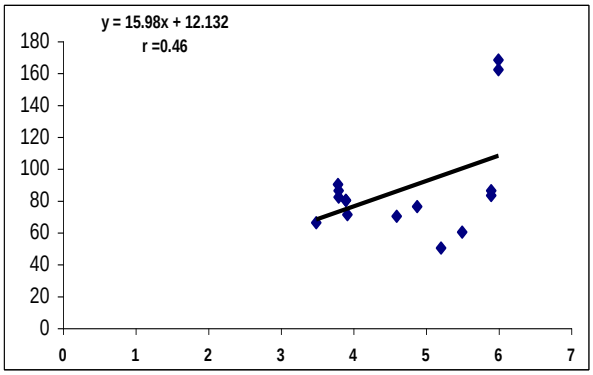
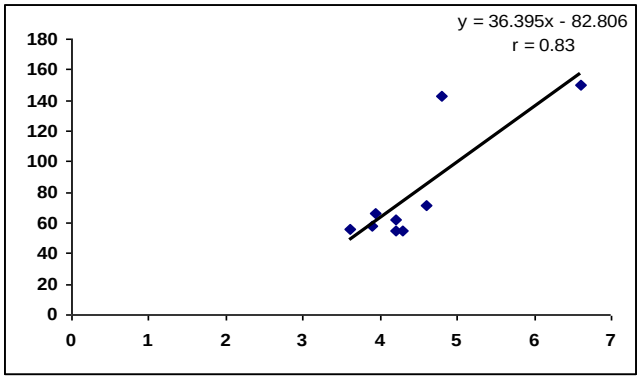


E



F

Fig (٣-١٠) The different correlations between TC.and CK activity in healthy controls and patients with diabetes mellitus.

A (Control male), B (Control female), C (Type I male), D (Type I female), E (Type II male), F (Type II female)

(٣-٥) The Effect of Diabetes on Triglyceride

Compared with healthy controls, triglycerides levels were found to significantly increased in sera of patients with different types of DM, as in Fig (٣-١١) and Table (٣-٦).

Fig (٣-٦) :-The levels of TG. in healthy controls and patients with diabetes mellitus.

	Sex	Mean	SD	TG		SE	٩٥٪ C.I		P	Sign
				Upper	lower		Upper	Lower		
Control	M	١.٤٥	٠.٢٧	٢	١.٢	٠.٠٥	١.٥٦	١.٤٢	٠.٠٠١	Sign
	F	١.٤٩	٠.٢٥١	٢	١.١٣	٠.٠٥٧	١.٦٢	١.٣٦	.٠٠٠١	Sign
Type ١	M	١.٨٢	٠.٦	٢.٩١	١.٣	٠.١١	٢.٨٧	١.٥٢٢	٠.٠٠١	Sign
	F	١.٥٣	٠.٤	٢.٣	١.٢	٠.١٣٧	١.٨	١.١٨	٠.٠٠١	Sign
Type ٢	M	٢.٠٤	٠.٩	٣١	١.١١	٠.٢٣	٢.٥٧	١.٥٠٩	٠.٠٠١	Sign
	F	١.٧٨	٠.٧٣	٢.٨	١.١	٠.٢٤	٢.٣	١.٢	٠.٠٠١	sign

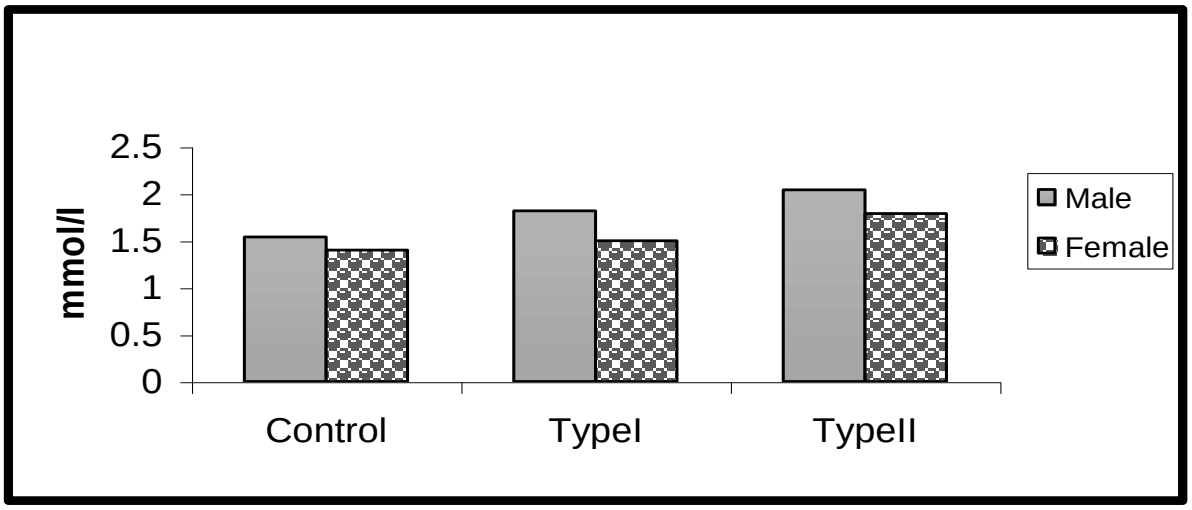
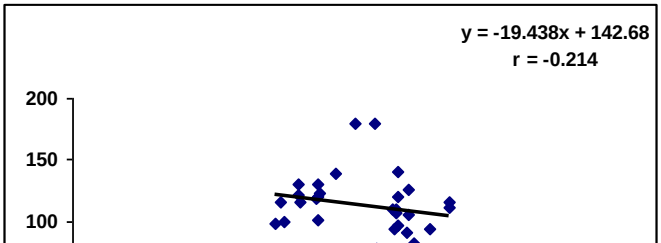
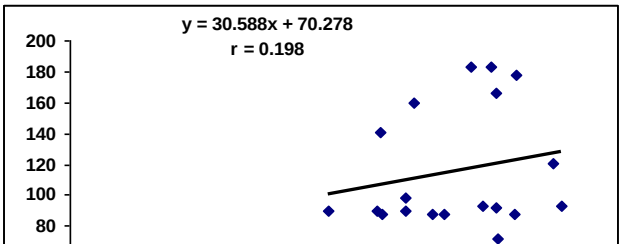


Fig (٣-١١) The levels of TG in sera of healthy controls and patients with diabetes mellitus

Hypertriglyceridemia is the most common lipid abnormality in DM. Insulin resistance and insulin deficiency may result because of change of lipoprotein lipase (LPL) activity. That may be due to increase hepatic production of triglycerides. Patients with well-controlled type II DM have normal TG levels. Hypertriglyceridema in type II is usually associated with obesity. The mechanism for acute, transient hyperglycemia remains unclear [٢٠٧]. Miles *et al* Indicate that a radio-labeled lipid emulsion can be used effectively to investigate the systemic and regional trafficking of LPL-generated glycerol and fatty acid in humans' [٢٠٩]. The Different Correlations between CK Activity and Triglyceride in Healthy Controls and Patients with Diabetes Mellitus are shown in Fig (٣-١٢).





A **B**

C **D**

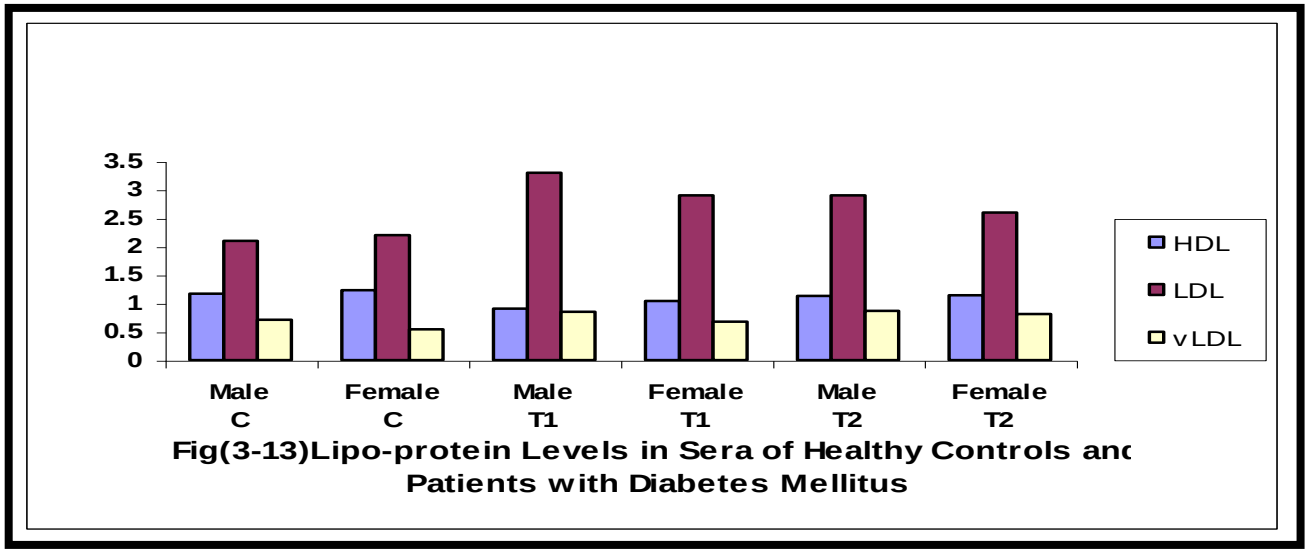
E **F**

Fig (٣-١٢) The different correlations between CK activity and triglyceride in healthy controls and patients with diabetes mellitus. A(Control male), B (Control female), C (Type I male), D (Type I female), E (Type II male), F (Type II female)

(٣-٦) The Effect of DM on Lipoproteins: -

The role of lipid abnormalities and the need for early detection of risk factors in insulin- resistance patients with or without diabetes is associated with cardiovascular disease [٢١٠]. The typical dyslipoproteinemia of type ٢ diabetes is characterized by increased VLDL, small ((dense)) LDL particles [٢١١]. The most important factor in DM is the size of LDL particle. The percentage of patients having small LDL is increased by at least two folds in type II DM [٢١٢].

Compared with healthy controls, HDL levels were found to significantly decrease in sera of patients with different types of DM, where as LDL, and vLDL levels were found to significantly increase in sera of patients with different types of DM, as shown in Fig (٣-١٣) and Table (٣-٧).



Fig(3-13)Lipo-protein Levels in Sera of Healthy Controls and Patients with Diabetes Mellitus

Table (٣-٧) Lipo-protein levels in sera of healthy controls and patients with diabetes mellitus.

		Sex	Mean mmol/l	SD	Upper	Lower	SE	٩٥٪ I.C		P	Sign
								Upper	Lower		
HDL	Control	M	١.١٧	٠.٠٩٩	١.٣	٠.٩	٠.٠١٧	١.٢٠٨	١.١٣	--	--
		F	١.٢٣٩	٠.١٥٩	١.٣٥	١.١١	٠.٠٩	١.٥٩	١.٣٩	--	--
HDL	T_1	M	٠.٩٠٥	٠.١٩٧	١.٢	٠.٧	٠.٠٣٥	٠.٩٨	٠.٨٢	٠.٠٠١	Sign
		F	١.٠٤	٠.١٢	١.٢	٠.٩٩٤	٠.٠٢١	١.٠٨٩	٠.٩٩٤	٠.٠٠١	Sign
HDL	T_2	M	١.١٣	٠.٢	١.٣٣	٠.٧٥	٠.٠٦	١.٢٨	٠.٩٥	٠.٠٠١	Sign
		F	١.١٤٧	٠.٣٩	١.٤	٠.٨٩	٠.٠١٩	١.١٨٧	١.٠٤	٠.٠٠١	Sign
LDL	C	M	٢.١	٠.٣٦	٢.٦	١.٤	٠.٠٦	٢.٢٩	٢.٠٠	--	--
		F	٢.٢	٠.٦	٢.٧	٢.٠	٠.١٤	٢.٧	٢.٠٠	--	--
LDL	T_1	M	٣.٣	٠.٨	٣.٧	٢.٠٠	٠.١٥٣	٣.٩	٢.٩	٠.٠٠١	Sign
		F	٢.٩	٠.٧	٣.٠	٢.٠	٠.٢٤	٣.٧	٢.٦	٠.٠٠١	Sign
LDL	T_2	M	٢.٩	٠.٨	٣.١	١.٩٧	٠.١٥	٣.٩	٣.٣	٠.٠٠١	Sign
		F	٢.٦	١.٠	٢.٩	٢.٠٥	٠.٣	٣.٠٠	٢.١١	٠.٠٠١	Sign
VLDL	C	M	٠.٧٠٩	٠.٠٥	٠.٨	٠.٥٣	٠.٠١	٠.٧٦	٠.٦٣٦	--	--
		F	٠.٥٤٥	٠.٠٤	٠.٦٥	٠.٣٨	٠.٠٧	٠.٦	٠.٤٩٧	--	--
VLDL	T_1	M	٠.٨٥	٠.١٣	٠.٩٨	٠.٦٨	٠.٠٢	٠.٩٤	٠.٧٠٤	٠.٠٠١	Sign
		F	٠.٦٨٨	٠.٠٧٦	٠.٩	٠.٥	٠.٠٢٥	٠.٨١٥	٠.٥٥٩	٠.٠٠١	Sign
VLDL	T_2	M	٠.٨٧٧	٠.١٥	١.١	٠.٥٥	٠.٠٣	٠.٩٨	٠.٦٨١	٠.٠٠١	Sign
		F	٠.٨١	٠.١٤	١.٥١	٠.٥١	٠.٠٤٨	٠٩٨	٠.٦٧	٠.٠٠١	Sign

Decreased HDL cholesterol levels are noticed in ٧٤.٦% of patients with type ١ diabetes and ٥٣% of patients with type II diabetes, especially in association with hypertriglyceridemia. Patients who used (Daonil) have (HDL) levels more than patients that used other drugs in NIDDM. Pervious studies have shown that vLDL triglyceride is not an important source of plasma FFAs.Wolf *et al* administered biosynthetic labeled vLDL to dogs and found that >٩٥٪ of vLDL-triglyceride fatty acids were taken up directly into tissues without traversing the plasma FFA pool. On the other hand, glycerol produced from VLDL by the action of LPL was released into the circulation [٢١٣],

determine lipoprotein subclass distribution by using nuclear magnetic resonance (NMR) spectroscopy. They found that NIDDM increased the large VLDL particle concentration, decreased in LDL size and decreased HDL size as a result of depletion of HDL particles. Most studies have concluded that oxidized low-density lipoprotein is responsible for atherosclerosis[٢١٤]. The macrophage engulfed oxidized LDL form foam cell. The accumulation of foam cells in sub endothelial space is due to endothelial cell injury and thereby the atherosclerotic was initiated [٢١٥,٢١٦]. LDL can be oxidized either in presence of transition metals, or in metal independent system.

The different correlations between lipo-protein and CK activity in control, type I and type II is shown in Fig (٣-١٤).

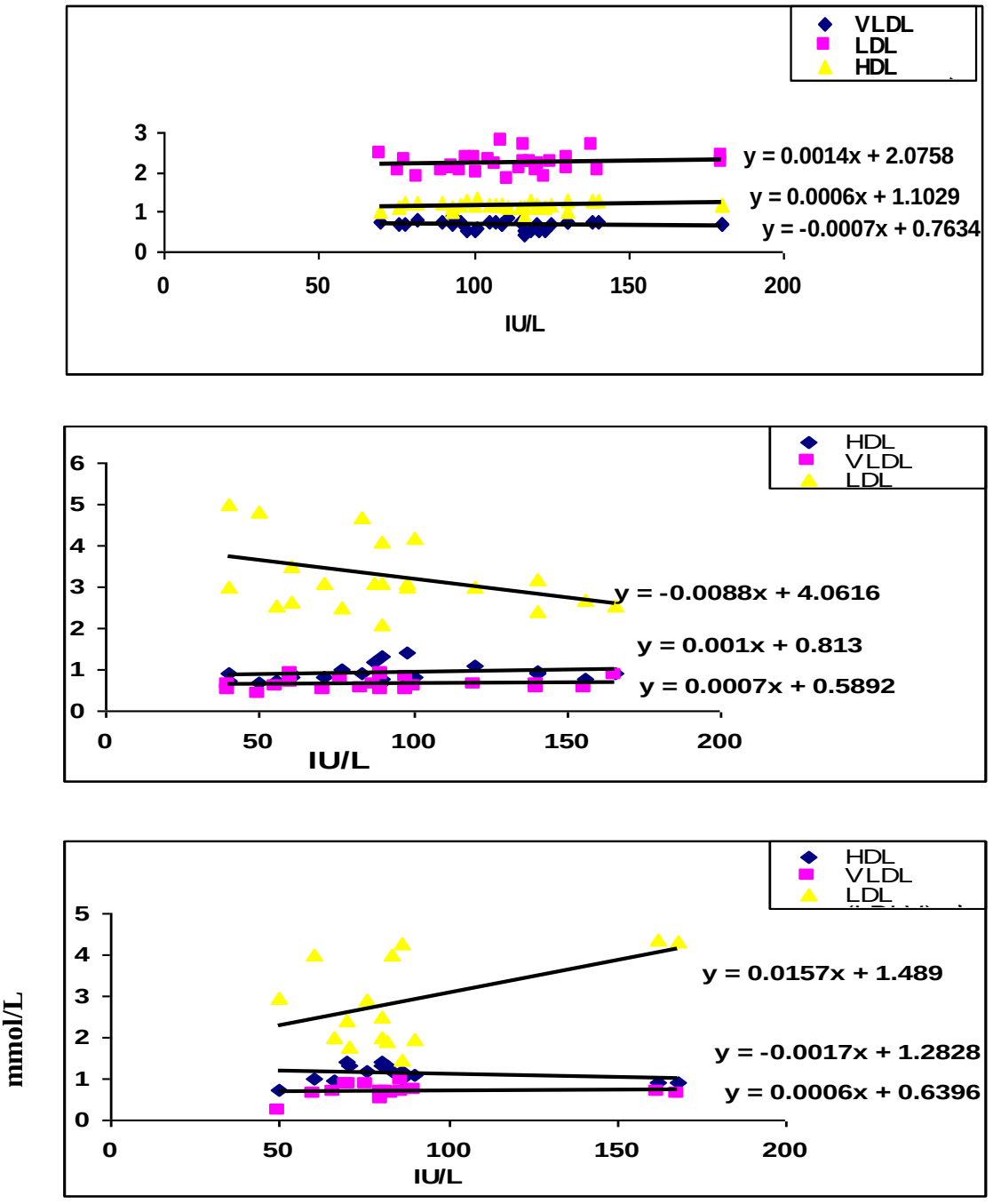
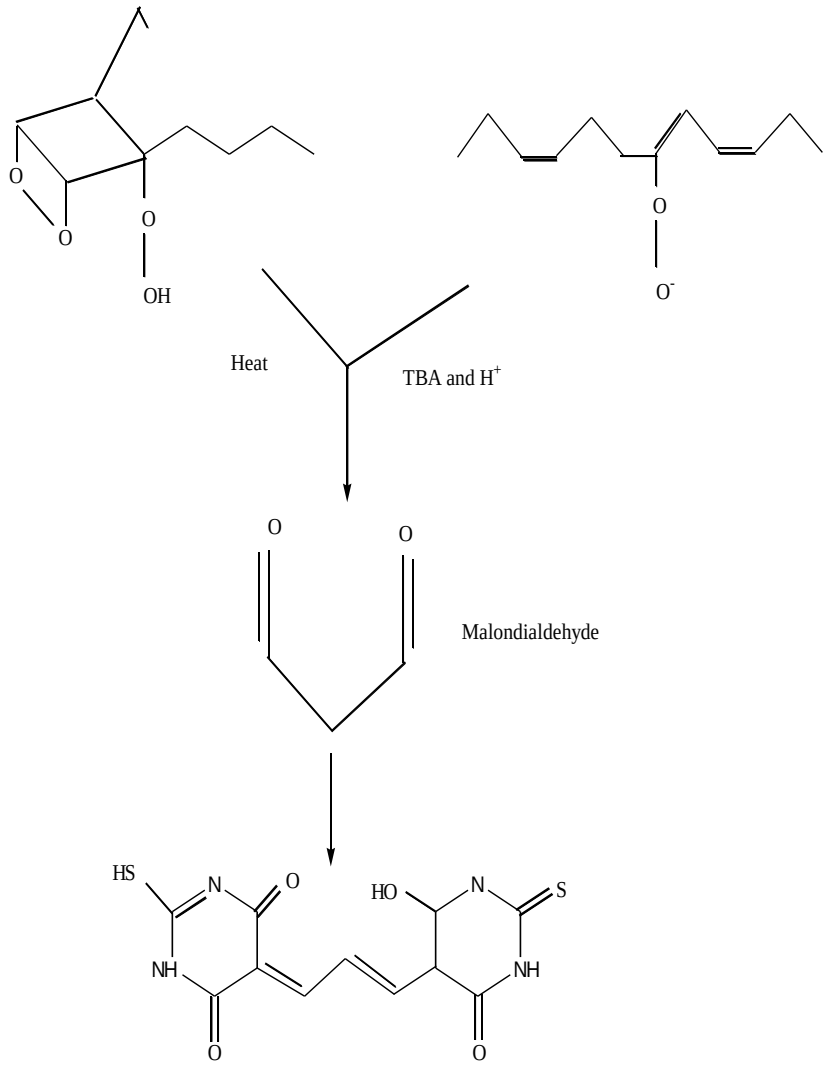


Fig (٣-١٤) The different correlations between lipo-protein and CK activity in control, Type I and type II. A (Control), B (Type I), C (Type II).

(٣-٧) Lipid Peroxidation

To estimate lipid peroxidation process, must be determining the product "Malondialdehyde" [٢١٧] as in fig (٣-١٣).



TBA Chromogen (absorbance 532 nm)

Fig (٣-١٥) Schematic diagram showing the principle of reaction for estimate Lipid peroxidation .

Table (٣-٨) The levels of MDA in healthy controls and patients with diabetes mellitus.

	Sex	Mean	SD	MDA(µmol/l)		SE	٩٥٪ I.C		P	Sign
				Upper	lower		Upper	Lower		
Control	M	١.٠٨	٠.٣٢	١.٦	٠.٩	٠.٠٥	١.١٩	٠.٩٥	--	--
	F	٠.٩٣	٠.٢٦٦	١	٠.٦	٠.٠٦	١.٠٦	٠.٨٠١	--	--
Type ١	M	١.٩٨	٠.٣٣	٢.٥	١.٦	٠.٥٩	٢.١١	١.٦٥	٠.٠٠١	Sign
	F	١.٩٤	٠.٣٤	٢.٣	١.٧	٠.١١٦٢	٢.٢	١.٦٨	٠.٠٠١	Sign
Type ٢	M	١.٩٥	٠.١٦٨	٢.٢	١.٧	٠.٠٤	٢.٠٥	١.٨٥	٠.٠٠١	Sign
	F	١.٨٥	٠.٣٢	٢.٣	١.١	٠.١٠٧	٢.٠٩	١.٦٠٧	٠.٠٠١	sign

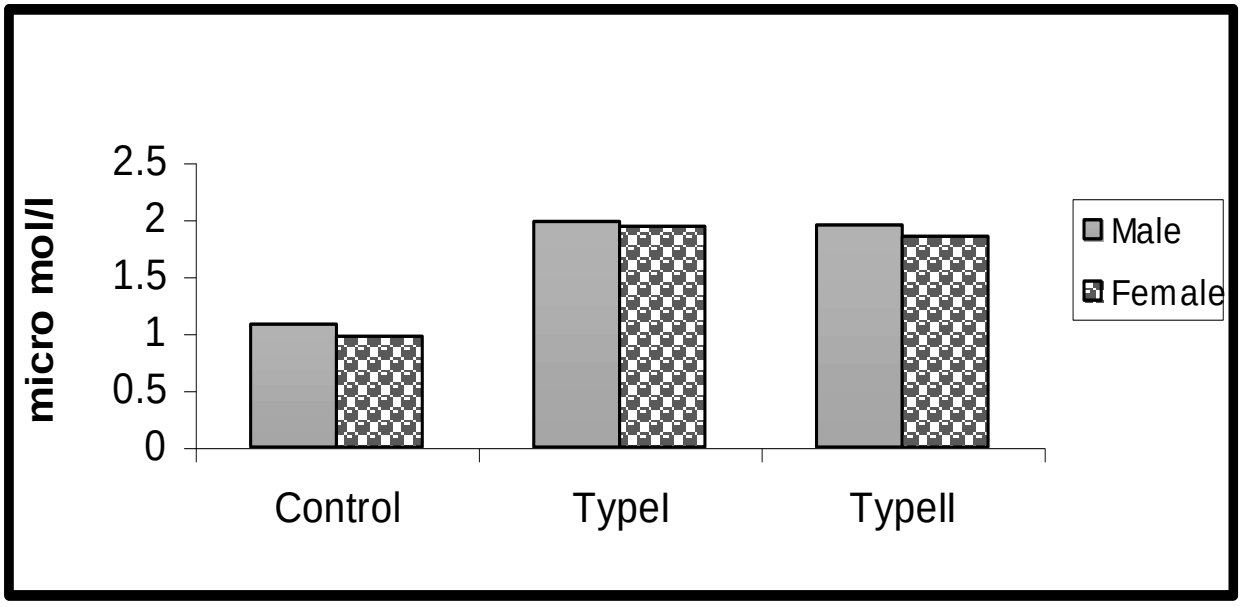


Fig (٣-١٦) The levels of MDA in sera of healthy controls and patients with diabetes mellitus

A higher level of malondialdehyde occurs because of the depletion of several forms of scavengers [٢١٨,٢١٩].

Lipid peroxidation is formed via mechanism composed from three steps (١) The first, initiation step is triggered by a reactive species as initiator by abstructing hydrogen and forming lipid alkyl radical.(٢) The second step is the propagation step by rapidly oxygen molecule and forming lipid peroxyl radical(٣) The third is the termination step which includes forming lipid hydroperoxide by abstracting hydrogen from a neighboring allyic bond.

The significant increase of lipid peroxidation in DM is associated with the increase of free radicals and the decrease of antioxidants. Free radicals are attached with lipid due to tissue damage [١٣٥].

$\dot{O}H$ Radical is generated according to the fenton reaction in which hydrogen peroxide reacts with metal such as $Fe^{٢+}$ or $Cu^{+٢}$ [١٣٧].

$$H_2O_2 + Fe^{+2} \rightarrow 2\dot{O}H + Fe^{3+}$$

Fenton Reaction

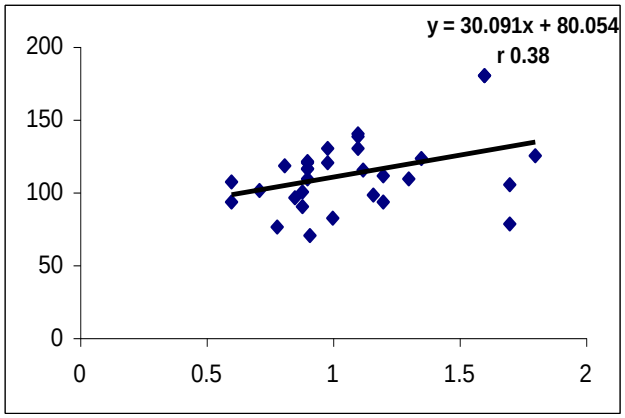
$\dot{O}H$ radicals generated according to the Haber Weiss reaction. This reaction involved reduction of Fe٣+ toFe+٢ by super oxide anion. The resultant $Fe^{+٢}$ contributes in the generation of $\dot{O}H$ as in fenton reaction.

$$\dot{O}_2^- + Fe^{3+} \rightarrow Fe^{2+} + O_2$$

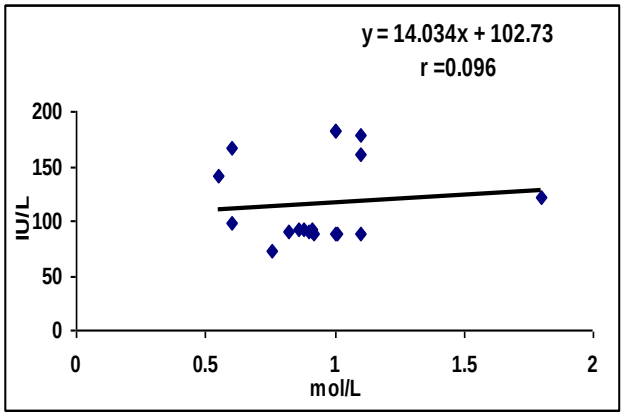
$$H_2O_2 + Fe^{+2} \rightarrow Fe^{3+} + \dot{O}H + O\bar{H}$$

Haber Weiss reaction.

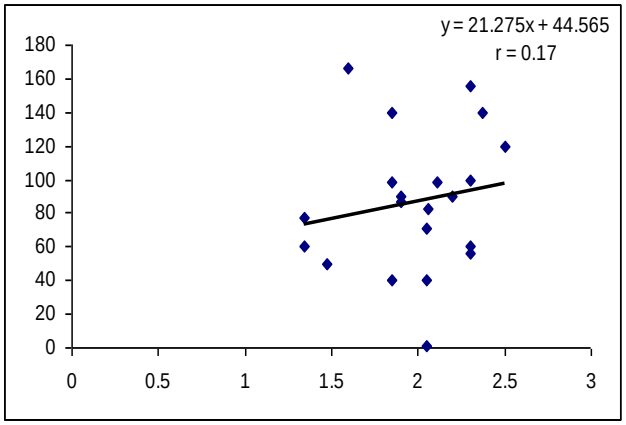
The Different Correlations between MDA Levels and CK Activity are shown in Fig (٣-١٧)



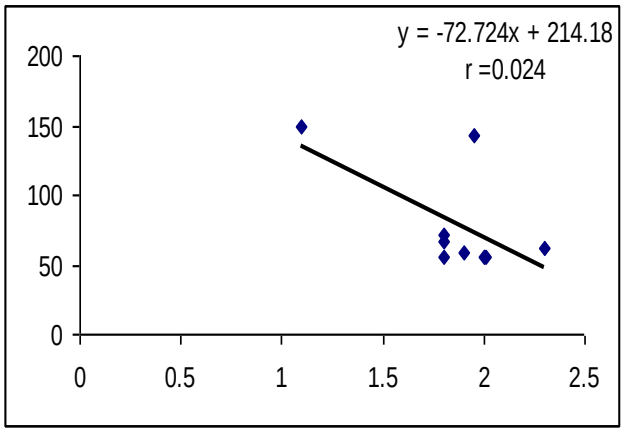
A



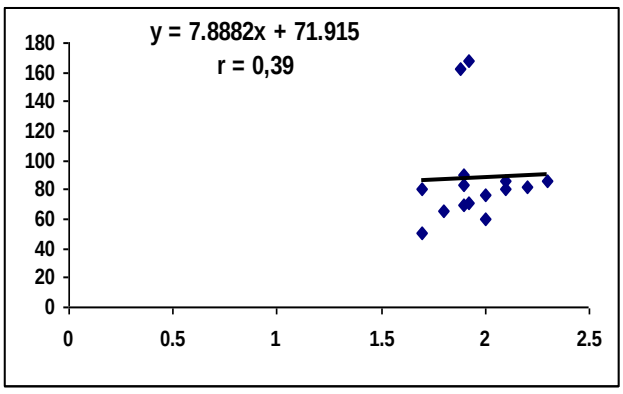
B



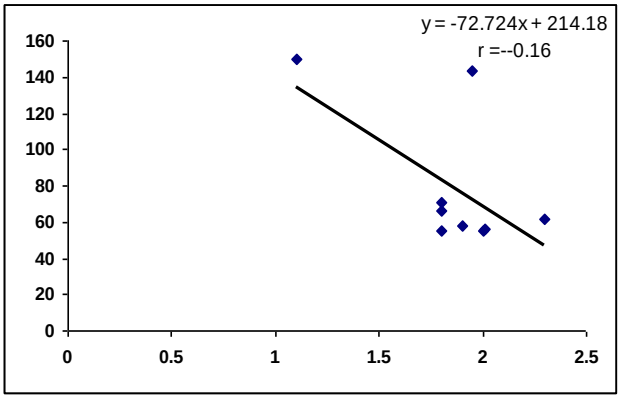
C



D



E



F

Fig (٣-١٧) The different correlations between MDA levels and CK activity. A) Control male), B (Control female), C (Type one male), D (Type one female), E (Type two male), F (Type two female).

(٣-٨) Conclusions: -

١- Diabetes mellitus decreases creatine kinase activity.

٢- Diabetes Mellitus is more effective on MM-CK and MB-CK than BB-CK.

٣- Glutathione and U.A levels are significantly decreased in sera of patients with different types of DM.

٤- A significant increase in serum total cholesterol and triglyceride levels was observed in-patients when compared with healthy controls.

٥- Diabetes decreases HDL levels and increases LDL and VLDL.

٦- Daonil increases HDL in-patients with type two-DM.

٧- Serum MDA concentrations show increase in patients with DM.

(٣-٩) Recommendations:

١- Study of the activity of CK in other types of diseases such as cancer and cardiovascular disease.

٢- Study of the effect of drugs used with DM on the levels of different kinds of antioxidants and isoenzyme of CK.

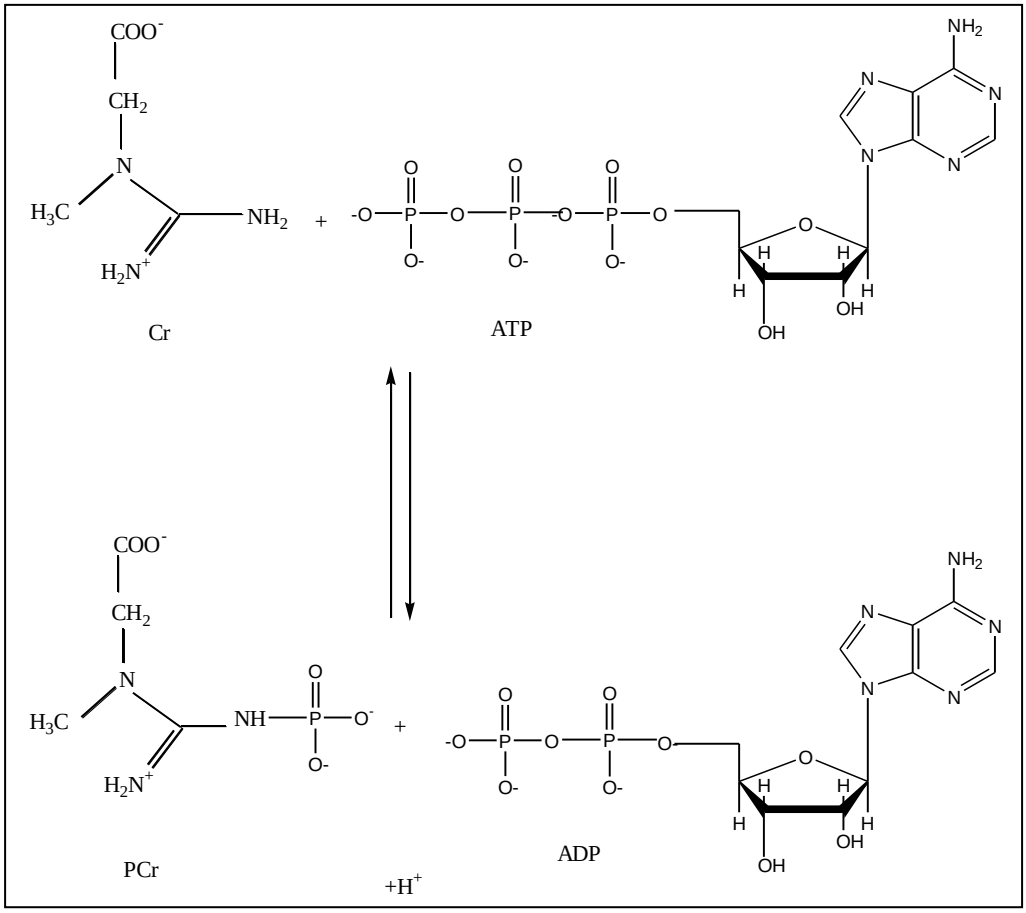
٣- Study of the effect of drugs that used to increase CK on the different tissues of patients with DM.

٤- Study of the relations between CK activity and transition elements such as Zn and Fe in-patients with different types of DM.

LIST OF ABBREVIATIONS

AA	ASCORBIC ACID
AMP	Adenosine mono phosphate
AGEs	Advanced glycosylated end products
apo	Apo lipo-protein
ATP	Adenosine triphosphat
B-Ck	Cytosolic Brain-type Ck isoenzyme
CK	Creatine kinase
CAT	Catalase
Cr	Creatine
Crn	Creatinine
Cys	Cystein
DHA	Dehydroascorbic acid
DTNB	-٬٥,٥Dithio Bis(٢-Nitrobenzoic acid
(GDM)	Gestational Diabetes Mellitus
DTT	Dithiothretol
GMP	Guanosine mono phosphate
GSH	Reduced glutathione
GSSG	Oxidized glutathione
$GS^{\cdot}$	Glutathyl radical
GS^{-}	Glutathione anion
HDL	High density lipoprotein
$HO^{\cdot}$	Hydroxyl radical
H_2O_2	Hydrogen peroxide
IMP	Inosine mono phosphate
IDDM	Insulin-dependant diabetes mellitus
$L^{\cdot}$	Lipid alkyl radical
LDL	Low density lipoprotein
$LO^{\cdot}$	Lipid alkoxyl radical
LOOH	Lipid hydroperoxid
M-Ck	Cytosolic muscle– type Ck isoenzyme
MDA	Malondialehyde
Mi-Ck	Mitochondrial Ck isoenzyme
NADPH	Nicotinamide adenine dinucleutide
NIDDM	NonInsulin-dependant diabetes mellitus
$O_2^{-\cdot}$	Superoxide anion

PI	Inorganic phosphate group
PGG$_{۲}$	Prostaglandin G$_{۲}$
PUFA	Ploy unsaturated fatty acid
RNA	Ribonucleic acid
RNS	Reactive Nitrogen Species
R.۱.P	Ribose –۱-phosphate
ROS	Reactive oxygen Species
SOD	Superoxide Dismutase
ROOH	Alkyl hydroperoxid
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substance
Tris	Tris (Hydroxymethylene)Aminomethane
UA	Uric acid
VLDL	Very Low density lipoprotein
XMP	Xanthosine mono phosphate


Cr
ATP
PCr
ADP
+H+

LIST OF FIGURES

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