# Biomarkers of Oxidative Stress and Antioxidant Status in Type 2 Diabetes: Importance in treatment

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# INTRODUCTION

- Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia which results from body's inability to produce insulin or use insulin to its full potential (Wold, et al., 2005; Kowluru and Chan, 2007).

- It is a lifelong progressive metabolic disease affecting more than 230 million people worldwide and this number is expected to reach 350 million by year 2025.

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INTRODUCTION

Risk of developing type 2 diabetes increases with age. Although age is not a modifiable risk factor for type 2 diabetes, data available so far indicate that life style choices can alter the progression of the disease which may be important for improving the overall quality of life for its sufferers.

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- Pathogenesis

  Several pathological processes are involved in the development of diabetes, which range from auto immune destruction of beta cells of pancreas causing insulin deficiency (type1 diabetes mellitus) to abnormalities that result in insulin resistance (type 2 diabetes mellitus) (Wold , et al.,2005).

- The impairment of insulin action in major target organs like liver and muscles is a common pathophysiological feature of type 2 diabetes (Kaku , 2010).

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- As a consequence to chronic hyperglycemia and insulin resistance , various long term complications develop.
- which includes both micro vascular and macro vascular like retinopathy with loss of vision, peripheral neuropathy with risk of amputation , autonomic neuropathy causing gastrointestinal, genitourinary, and cardiovascular symptoms and nephropathy causing kidney failure (Rahimi , et al.,2005 ;Wold, et al.,2005).

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- The development of the disabling chronic complications of diabetes mellitus has been attributed to oxidative stress (Opara , 2002), which results due to chronic hyperglycemia, dyslipidemia and elevated fatty acids in circulation (Kuroki, et al., 2003).

- Oxidative stress is an unavoidable consequence of life in  an atmosphere which is oxygen-rich (Davies,2000).



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Oxidative stress is a normal phenomenon in the body under normal conditions, the physiologically important intracellular levels of reactive oxygen species (ROS) are maintained at low levels by various enzyme systems participating in the in vivo redox homeostasis.

# Contd

Generation of reactive oxygen species is a normal immunologic response of the body to destroy invading pathogens (respiratory burst reactions)



oxidative stress can be viewed as an imbalance between the pro-oxidants and antioxidants in the body.



- In pro-oxidant/antioxidant homeostasis toxic reactive oxygen species (ROS) are generated by several mechanisms
- ROS include the following:
- hydrogen peroxide,
- organic hydro peroxides,
- nitric oxide, superoxide and
- hydroxyl radicals

# Prooxidants

## Exogenous

- Pathogens ,Drugs, Toxicants

(**Bacteria, virus, Fungus, Parasites**)

- Dietary ingredients

(**Lipids, Carbohydrates, Highly processed foods, Antioxidants)**

- **Environmental pollution**

**(Transition metals, pesticides, drug residues)**

- **Climate**

## Endogenous

- Endogenous metabolites
- Drug metabolites
- Cellular metabolites
- Ion flux
- Pathophysiology
- Ischemia

- Most of the free radicals are produced at low levels during normal physiological conditions and scavenged by endogenous antioxidant system

- Oxidative stress occurs when production of reactive oxygen species(ROS)& reactive nitrogen species(RNS) exceeds the capacity of cellular antioxidants defenses to remove these toxic species

(Johansen, etal.,2005;Limón-Pacheco and Gonsebatt , 2009).

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- **Evidence is accumulating steadily, supporting the general importance of oxidative damage of tissue and cellular structural and functional components as a primary or secondary causative factor in many different human diseases including diabetes and their deleterious effects.**

# Hyperglycemia causes tissue damage

*Increased flux of glucose and other sugars through the polyol pathway;

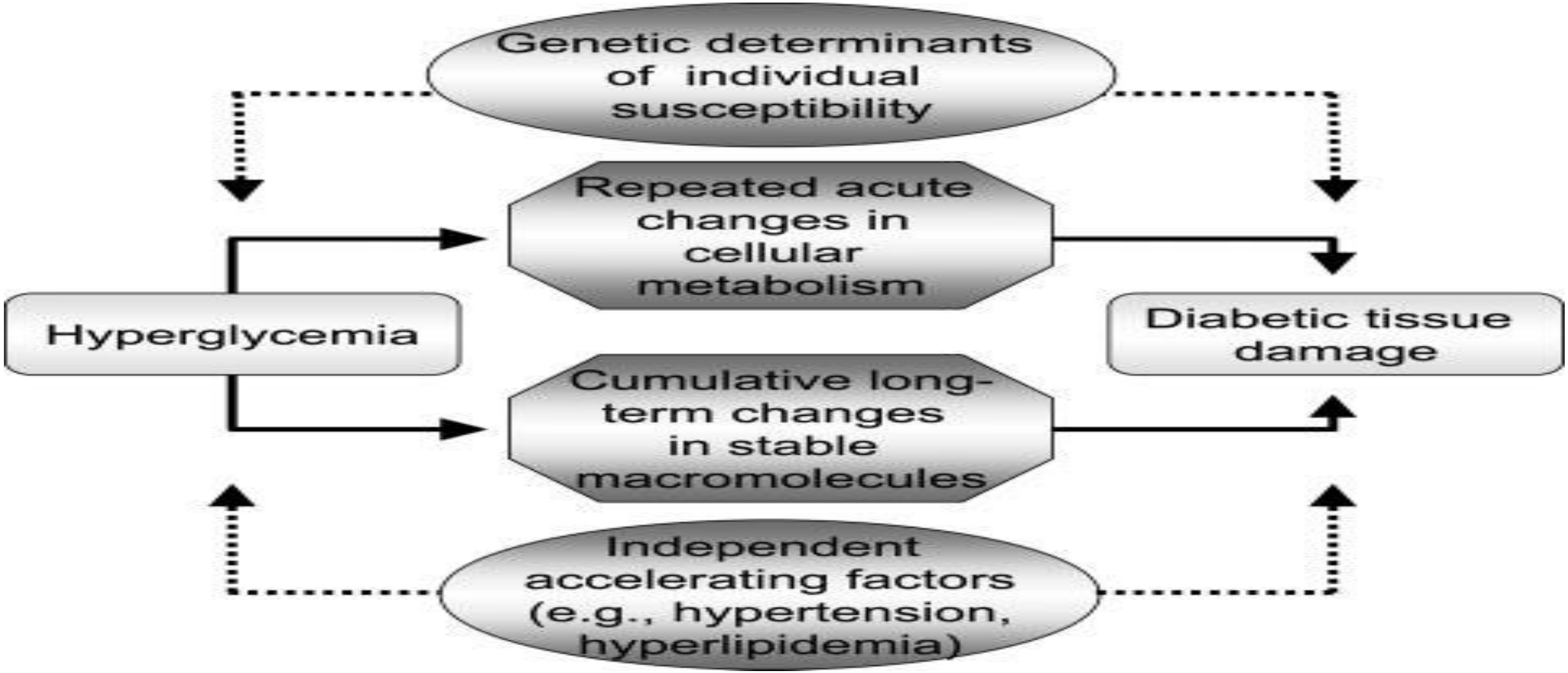
* Increased intracellular formation of AGEs (advanced glycation end products);

• Increased expression of the receptor for AGEs and its activating ligands;

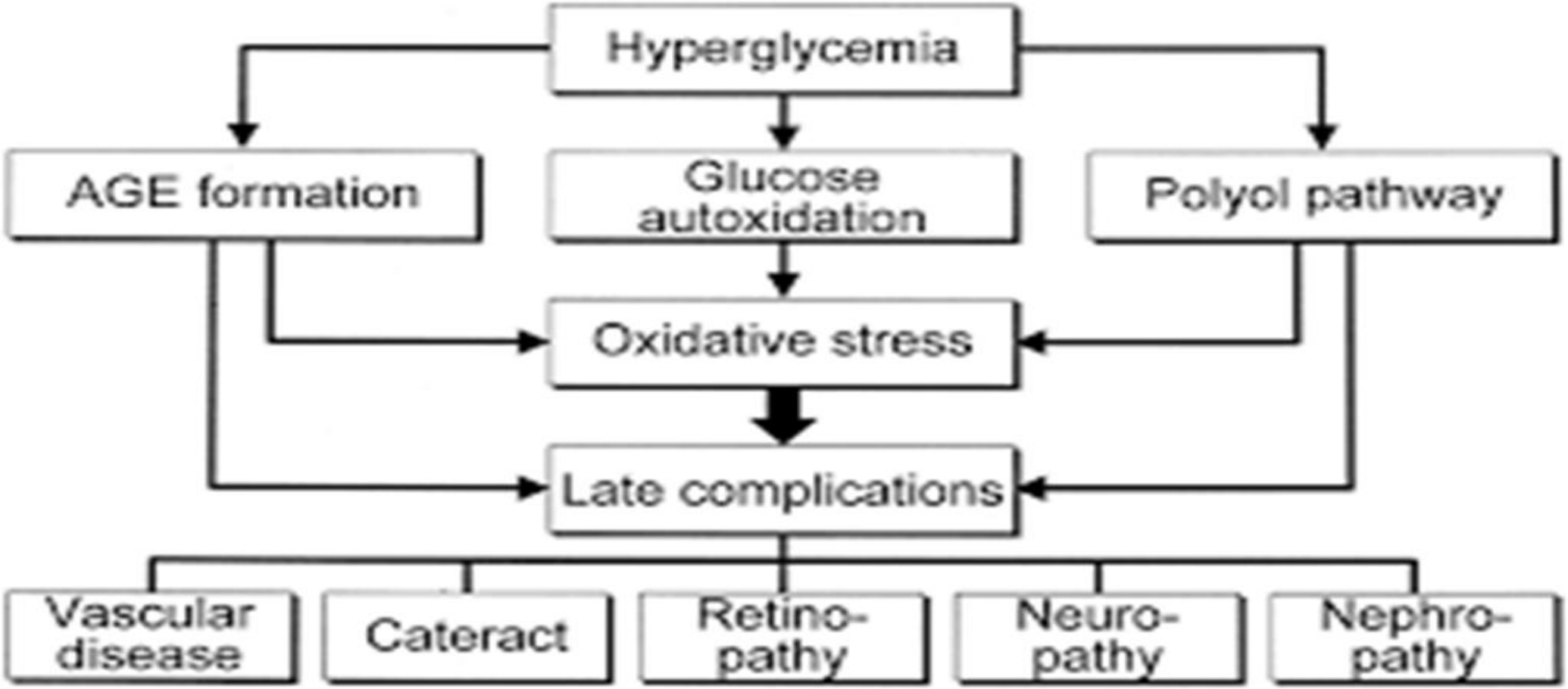
* activation of protein kinase (PK)C isoforms; and

* overactivity of the hexoseamine pathway

**General features of hyperglycemia-induced tissue damage.**
(Brownlee)



# Oxidative stress in Diabetes

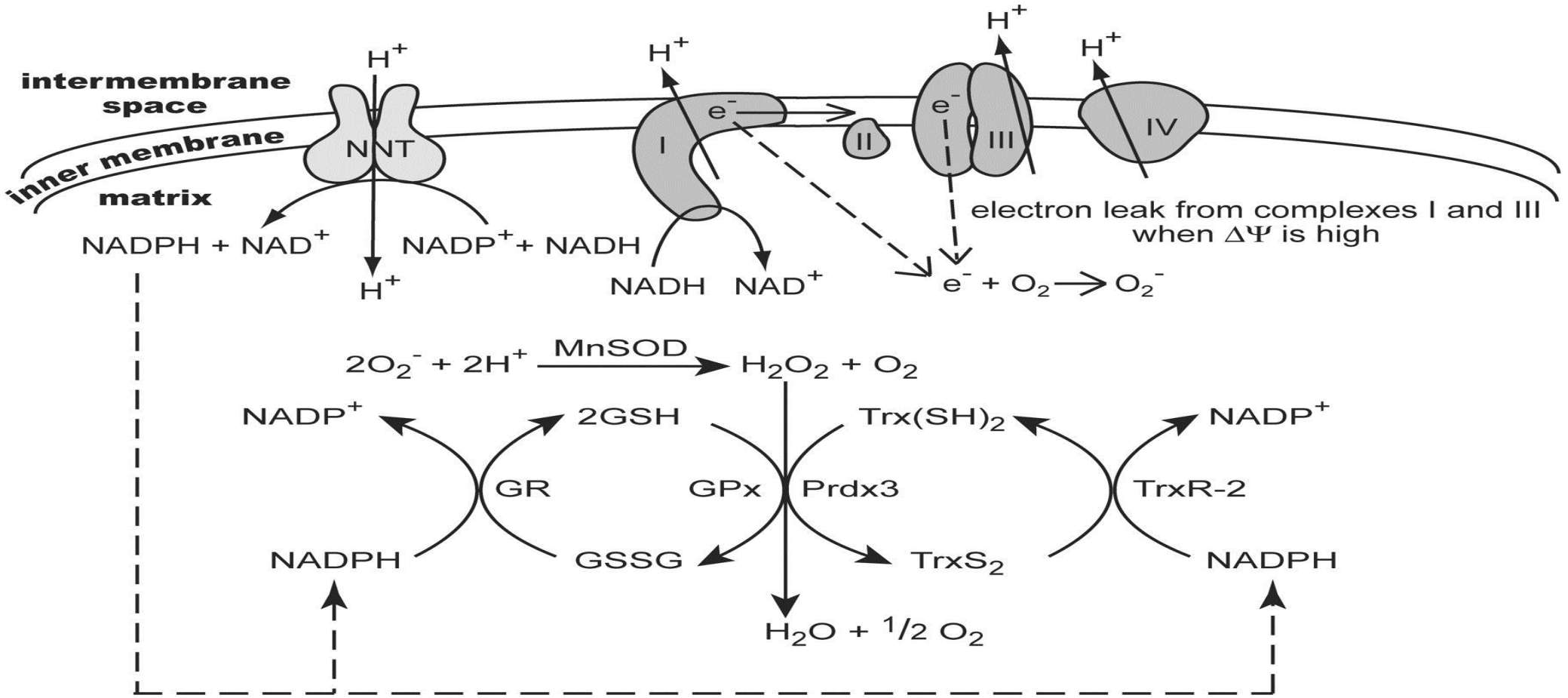


# Hyperglycemia also inactivates endothelial nitric oxide synthase and prostacyclin synthase.

**Through these critical defective pathways**

- **Increased intracellular reactive oxygen species (ROS) are formed and cause defective angiogenesis in response to ischemia,**
- **Activate a number of proinflammatory pathways, and cause long-lasting epigenetic changes that drive persistent expression of proinflammatory genes after glycemia is normalized (“hyperglycemic memory”).**

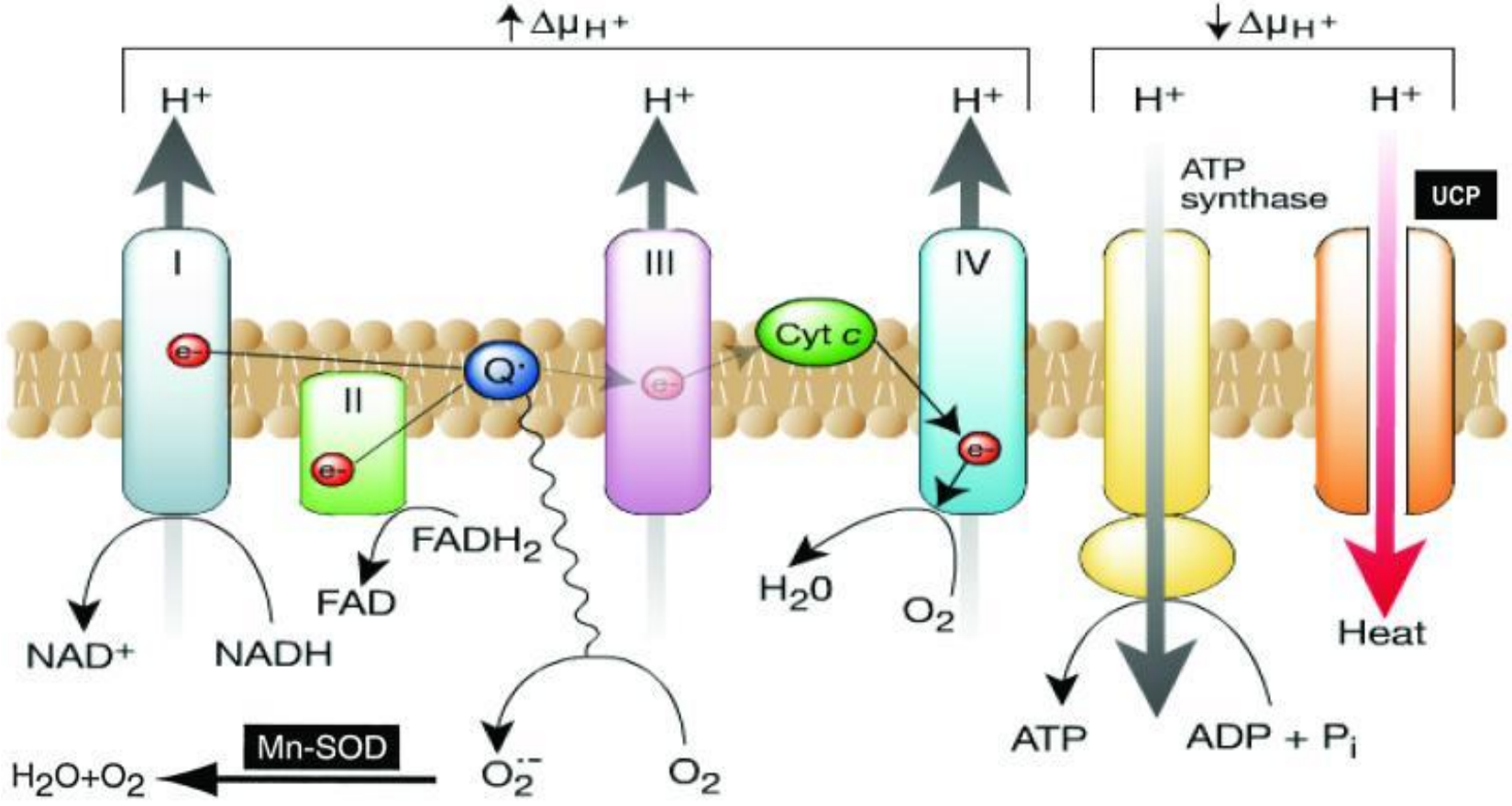
# Hyperglycemia-Induces Mitochondrial Superoxide Production



- Pathogenic mechanisms described in previous slides originate from a single hyperglycemia-induced process, namely overproduction of superoxide by the mitochondrial electron-transport chain.
- Superoxide ($O_2^{\cdot-}$) is the initial oxygen free radical formed by the mitochondria, which is then converted to other more reactive species that can damage cells in numerous ways.



# Production of ROS by mitochondrial ETC (Brownlee)



# Over Expression of MnSOD or Calalase is cardio protective

- Superoxide production may amplify the damaging effect of hyperglycemia by redox changes, NADPH oxidases and uncoupled eNOS.
- overexpression of MnSOD or catalase protects cardiac mitochondria from oxidative damage, improves respiration, and normalizes mass in diabetic mitochondria. MnSOD also prevents the morphological changes in diabetic hearts and completely normalizes contractility in diabetic cardiomyocytes

# Over Expression of MnSOD or Calalase is protective to vascular endothelium

- In endothelial cells increased MnSOD or UCP-2 expression inhibits both hyperglycemia and fatty acid–induced inactivation of the antiatherosclerosis endothelial enzyme prostacyclin synthase by nitration in diabetes. Overexpression of either MnSOD and UCP-1 also prevents inhibition of eNOS activity by these metabolites.

# Antioxidant supplementation

- Experimental evidences suggest antioxidants like N acetyl cysteine (NAC), Vitamin C, Vitamin E and α Lipoic acid supplementation to be effective in reducing complications indicating that antioxidant intake through diet or supplementation is beneficial in reducing the development of diabetic complications.

# Resveratrol of Redwine (RES) (trihydroxystilbene)

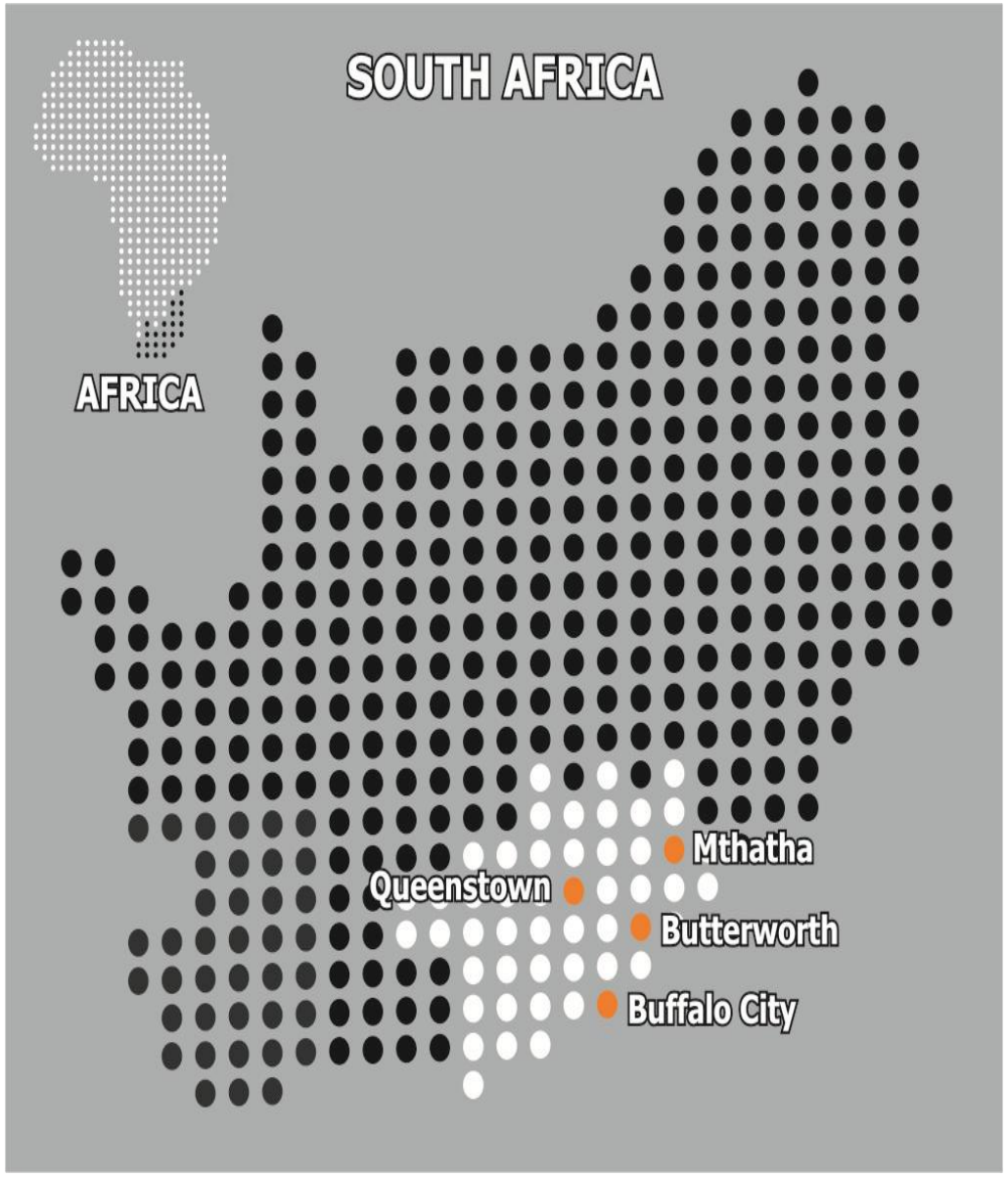
- Is a good example of a dietary polyphenol. The beneficial effect of RES has been attributed to its ability to reduce oxidative stress. Studies have shown an increase in antioxidant enzyme activities following exposure to RES including the induction (upregulation) of mitochondrial superoxide dismutase (MnSOD)

# Biomarkers of Oxidative Stress and Antioxidant Status in Type 2 Diabetes: A study among African diabetic patients on treatment

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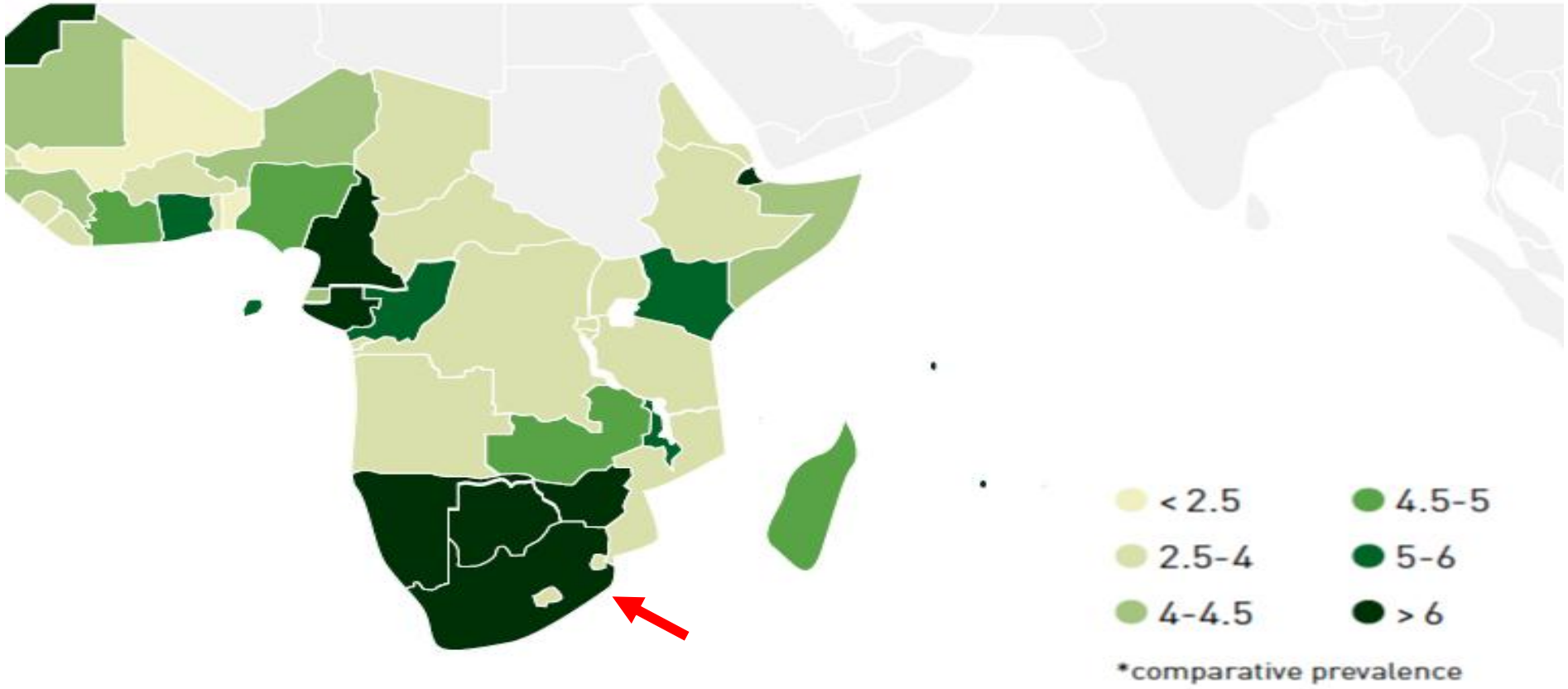

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# Prevalence estimates for diabetes in Africa, International Diabetes Federation 2011

**3.1. Prevalence*(%) estimates of diabetes (20-79 years), 2011, Africa Region**



- 3,3 million South Africans are diabetic and T2DM accounts for 90% of the South African diabetic population
- A Statistics SA report released in 2013 shows a gradual increase of mortality rate of diabetes from 3.3% to 3.9%, one of three diseases with gradual increase in mortality

## Aim of study:

- To determine oxidative stress and total antioxidant status in type 2 diabetic patients in Mthatha region of the Eastern Cape province of South Africa.
- The study was approved by the Walter Sisulu University Health Research Ethics and Biosafety Committee (Protocol number 012/12)

Research participant recruitment:

* A cross sectional observational study was conducted on 57 (33 F & 24 M) South African type 2 diabetic patients without diabetic complications attending selected diabetic clinics
* 41(26F&15M) healthy non- diabetic volunteers served as controls
- age group of the research participants – (35- 75yrs)

*Control participants were selected randomly from the general population keeping in mind the selection criteria.

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# Material and Methods:contd

Methods:

- Plasma glucose, glycated hemoglobin and lipid profile were measured by standard routine methods using ROSCHE COBBAS 6000 chemical auto analyzer by NHLS(National Health Laboratory Services) of NMAH(Nelson Mandela Academic Hospital).
- The Total Antioxidant level in serum was measured using commercial kit from Sigma Aldrich by ABTS method. Thiobarbituric acid reactive substances and SOD enzyme was measured using Cayman assay kits, USA (colorimetric method) and Oxidized LDL was measured by ELISA technique using Mercodia kit.
- BIO-TEK $KC_4$ AUTOREADER was used for all the above analysis.

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Statistical Analysis:

- Statistical analysis was performed using IBM Statistical package for the Social Sciences (SPSS Inc, Chicago, IL, USA version 23,) .
- Data are expressed as mean ± SD
- students- t test for normally distributed data and non parametric Mann-Whitney U test was done for parameters which did not follow normal distribution.
- All P values were two-tailed, and values of less than 0.05 were considered statisticaly significant.
- Bivariate correlations were performed using Spearman rank correlation to analyze relationships between continuous variables.

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- Results :contd
- Table 1 shows the information about the general characteristics of the participants.

**Table 1: Demographic characteristic of control and diabetic patients.**

| Parameters | Control Group, N=41 | Diabetic Group, N=57 | p- value |
|---|---|---|---|
| Age(years) | 54.24 ± 6.05 | 56.61 ± 7.59 | 0.101 |
| Weight(Kg) | 76.8 ± 15.9 | 83.4 ± 17.7 | 0.065 |
| Height(cm) | 159.4 ± 6.20 | 162.1 ± 7.87 | 0.057 |
| BMI($Kg/m^2$) | 30.4 ± 7.28 | 31.6 ± 6.49 | 0.378 |
| Duration of diabetes | - | 6.87±6.34 | |

Values are given as mean ± S.D (Standard Deviation), BMI: body mass index.

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# Results :contd

**Table 2: Comparison of biochemical parameters between control and diabetic groups.**

| Parameters | Control Group N=41 | Diabetic Group N=57 | p- value |
|---|---|---|---|
| FBS(mmol/L) | 4.89±0.53 | 8.70 ±3.76 | 0.0005*** |
| HbA1c (%) | 5.88±0.62 | 8.77±2.15 | 0.0005*** |
| Hemoglobin (gm %) | 14.1±1.49 | 14.1 ±1.44 | 0.854 |
| Total Cholesterol(mmol/L) | 4.70±0.92 | 4.51 ±1.23 | 0.260 |
| Triglyceride(mmol/L) | 1.21±0.50 | 1.59 ±0.85 | 0.031* |
| HDL Cholesterol(mmol/L) | 1.40±0.41 | 1.21 ±0.44 | 0.008** |
| LDL Cholesterol(mmol/L) | 2.72±0.88 | 2.57±1.03 | 0.461 |

Values are given as mean ± S.D (Standard Deviation), FBS: fasting blood sugar; HbA1c: Glycated hemoglobin.*p<0.05, **p<0.005, ***p<0.001 shows significant difference.

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# Results :contd

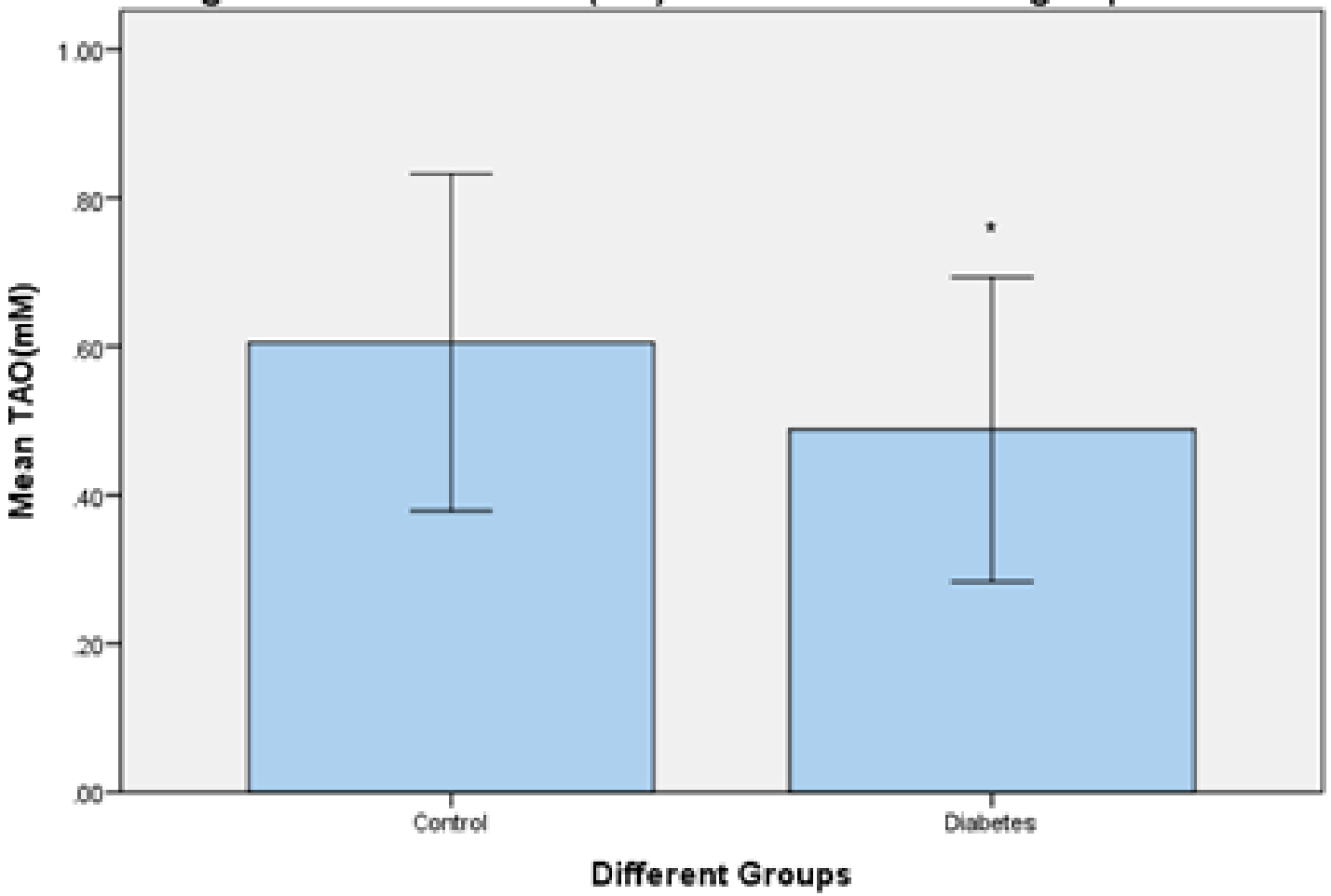
**Table 3: Antioxidant Status and lipid per oxidation in control and diabetic groups**

| Parameters | Control Group, N=41 | Diabetic Group, N=57 | p- value |
|---|---|---|---|
| Total antioxidant (mM) | 0.60±0.22 | 0.48±0.20 | 0.010* |
| SOD (U/ml) | 3.65±1.51 | 4.13±1.47 | 0.096 |
| TBARS(uM) | 3.60±1.65 | 4.70±1.86 | 0.004** |
| Oxidized LDL(U/L) | 81.4±32.0 | 101.4±44.5 | 0.022* |

Values are shown as mean ± S.D (Standard Deviation), SOD: superoxide dismutase;
TBARS: Thiobarbituric acid reactive substances.*p<0.05, **p<0.005 shows statistical significance.

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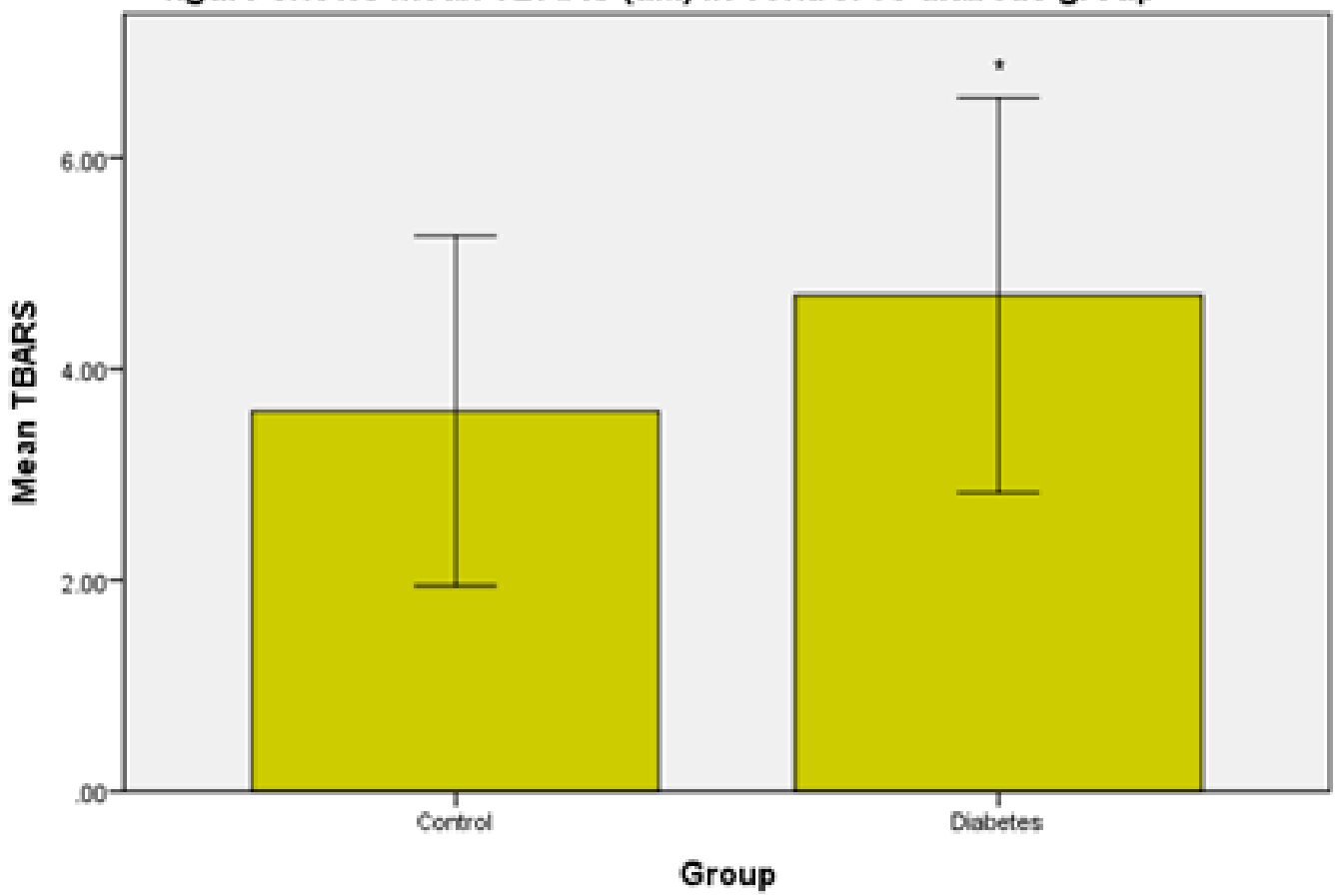
figure shows mean TAO (mM) in control vs diabetic group




*p<0.05 between control and diabetic group

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figure shows mean TBARS (uM) in control vs diabetic group

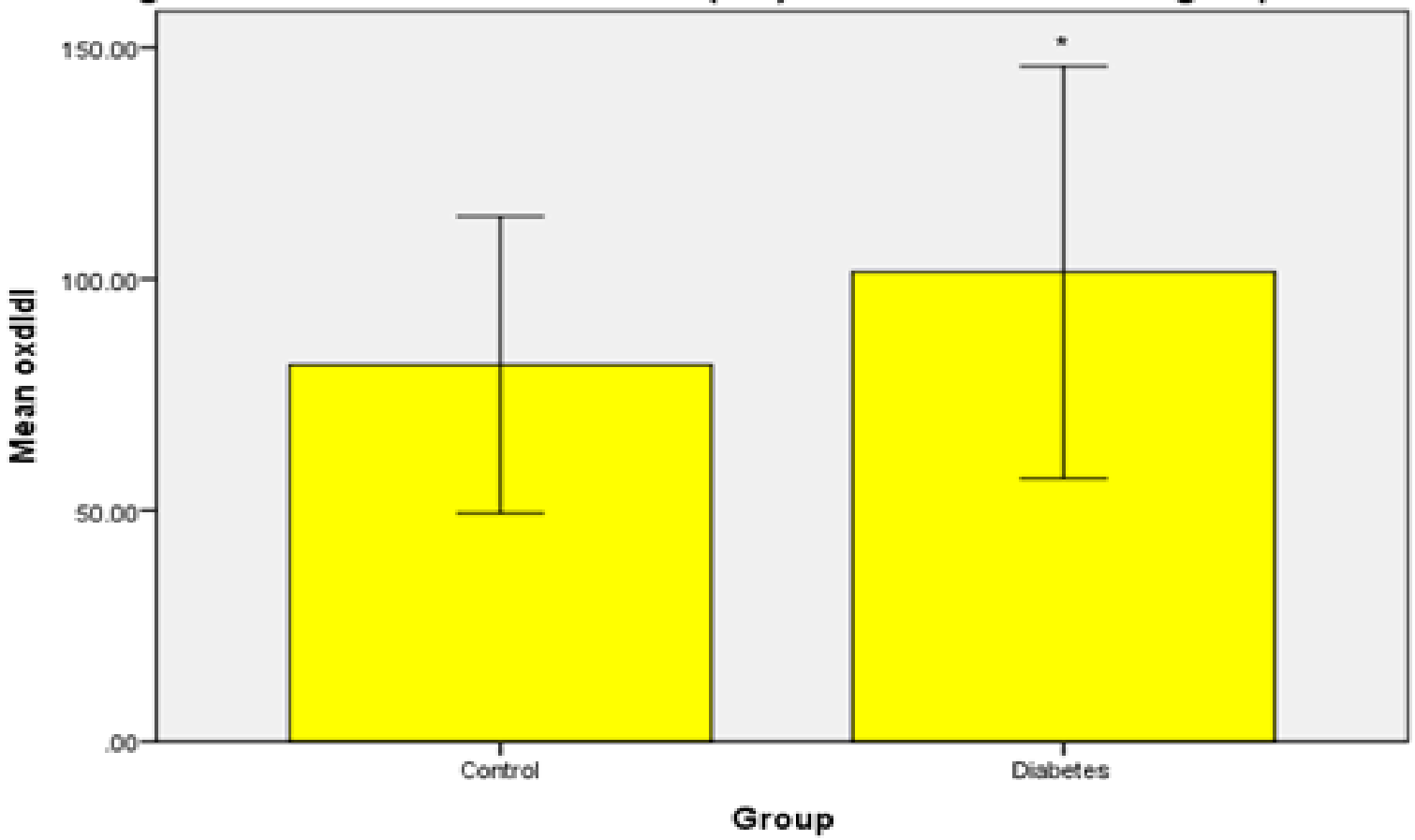



*p<0.005 between control and diabetic group

Error bars: +/- 1 SD

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figure shows mean oxidized LDL (U/L) in control vs diabetic group




*p<0.05 between control and diabetic group

Error bars: +/- 1 SD

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➢Results :Bivariate correlations done using Spearman rank correlation showed significant negative correlation relationships between TAO level and FBS, HbA1c, and SOD levels as shown in table 4 below.

**Table 4: Correlation of Total antioxidant level with FBS, HbA1c, TBARS, Oxidized LDL and SOD levels in study group.**

| parameters | Correlation coefficient | p- value |
|---|---|---|
| FBS(mmol/L) | r = -0.220 | 0.034* |
| HbA1c (%) | r =-0.277 | 0.008* |
| TBARS(uM) | r = -0.158 | 0.126 |
| Oxidized LDL(U/L) | r =-0.202 | 0.051 |
| SOD(U/ml) | r =-0.301 | 0.003** |

*p<0.05, **p<0.005 shows statistical significance.

# Results :contd

- HDL cholesterol also showed significant negative correlation between fasting blood glucose , glycated hemoglobin and triglyceride levels as seen in table 5.

**Table 5: Correlation of HDL cholesterol with FBS, HbA1c and Triglyceride levels in study group**

| parameters | Correlation coefficient | p-value |
|---|---|---|
| FBS(mmol/L) | r =-0.251 | 0.014* |
| HbA1c (%) | r= -0.289 | 0.005** |
| Triglyceride(mmol/L) | r=-0.405 | 0.0005*** |

*p<0.05, **p< 0.005 ,***p<0.0005 shows statistical significance.

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- Results :contd
- Table 6 shows significant positive correlation between TBARS and fasting blood glucose and glycated hemoglobin levels.

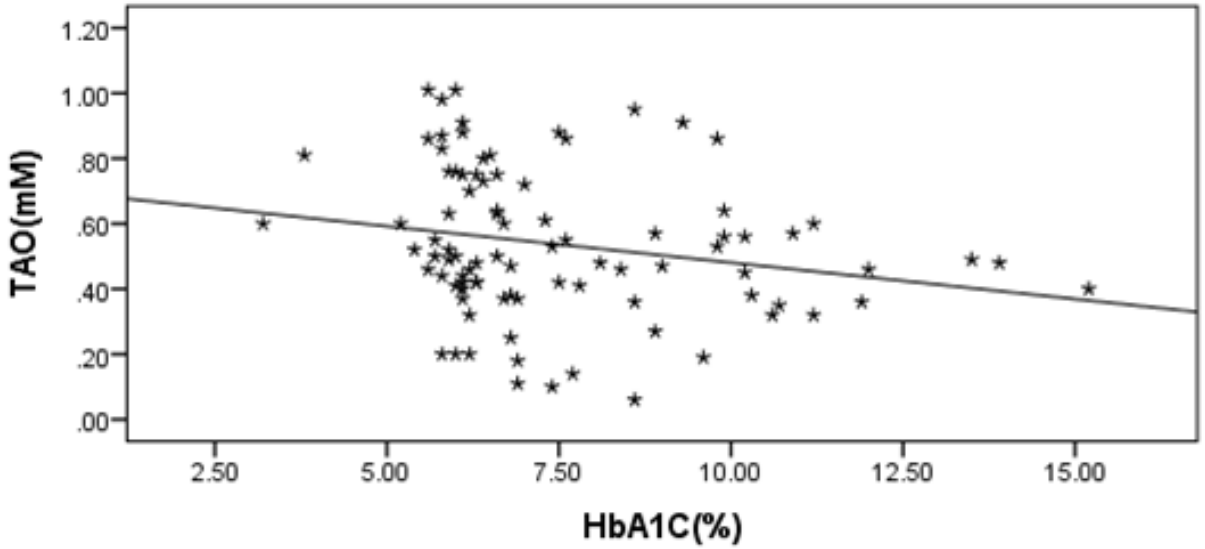
**Table 6 ; correlation between TBARS levels with FBS and glycated hemoglobin levels in study group**

| parameters | Correlation coefficient | p-value |
|---|---|---|
| FBS(mmol/L) | r=0.255 | 0.012* |
| HbA1c (%) | r=0.289 | 0.005** |

*p<0.05, **p<0.005 shows statistical significance.

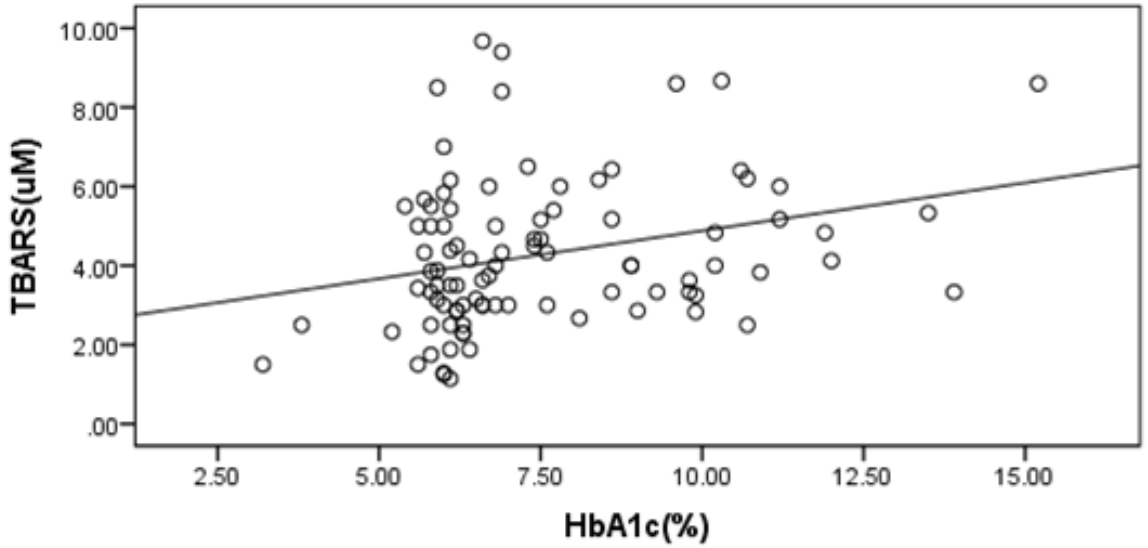
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**figure shows relationship between TAO VS HbA1C in study group**




Spearman Correlation coefficient ( r)= - 0.277, p<0.05

**figure shows relationship between TBARS VS HbA1C in study group**

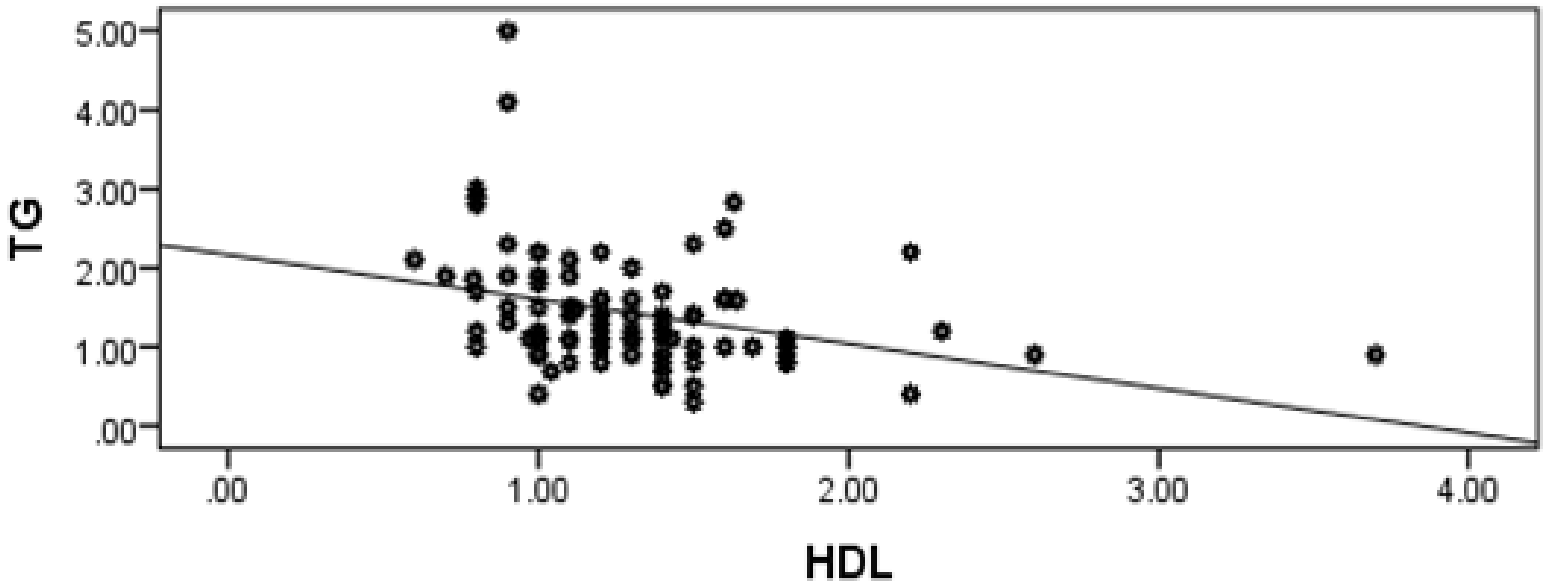



Spearman Correlation coefficient ( r)= 0.289, p<0.005

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figure shows relationship between HDL cholesterol VS Triglycerides in study group




Spearman Correlation coefficient ( r)= - 0.405, p<0.0005

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## Discussion :contd

- In the present study there was marked increase in HbA1c and FBS in diabetic patients as compared to control population indicating excessive glycosylation of hemoglobin as reported by other studies(Benrebai, et al.,2008;Kharroubi,et al., 2015;Pasupathi,et al.,2009;Merzouk,et al., 2004;Nourooz-Zadeh,et al.,1997 ).
- lipid profile showed serum triglycerides higher in diabetic patients and HDL cholesterol was lower in diabetic group as compared to control group indicating presence of dyslipidemia in diabetic patients.

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## Discussion :contd

- Hypertriglyceridemia and reduced plasma high density lipoprotein (HDL) is commonly present in T2DM as seen in different studies by(Pereira, et al.,2008;Kharroubi,et al.,2015; Pasupathi,et al.,2009).
- Significant negative correlation was observed between HDL cholesterol and serum triglyceride, plasma glucose and glycated hemoglobin.
- So with poor glycemic control ,HDL cholesterol decreases.

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## Discussion :contd

- The observed increase in TBARS levels in our diabetic patients are thought to be due to increased production of lipid peroxides and liberation in circulation and is consistent with previous studies(Benrebai, et al.,2008; Jamuna Rani and Mythili,2014;Mahboob,et al.,2005;Maharjan,et al.,2008).
- In our study increased LDL oxidizability is seen in diabetic patients. Lipid profile alter in this patients and can influence susceptibility of LDL to oxidation. HDL inhibits the oxidative modification of LDL and its reduction in diabetic patients can cause more oxidation of LDL.

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# Discussion :contd

- Decreased TAO status in diabetic patients could be due to increased oxidative stress as seen by increased lipid peroxidation as well as the excess utilization of antioxidants against oxidative stress to minimize the damage.
- Negative correlation between total antioxidant status and poor glycemic control (FBS, HbA1c) shows increase in oxidative stress due to uncontrolled hyperglycemia leading to depletion of antioxidants levels. Measurement of total antioxidant can be a marker for glycemic control.
- TBARS levels increase with increase in levels of plasma glucose and glycated hemoglobin levels as shown by a positive correlation, which is in accordance with the studies of Gupta and Chari (2006) and Kesavulu (2000).

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- In conclusion, results from the above study suggest that hyperglycemia in type 2 diabetes mellitus cause oxidative stress and decreased total antioxidant status
- Total antioxidant status could be an early biomarker for routine monitoring and evaluation of diabetic patients.
- Early intervention and proper diet rich in antioxidants can reduce the risk of developing debilitating complications and increase the longevity and quality of life of diabetic patients.

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# Thank you

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