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Impact of the level of adherence to the Mediterranean diet on metabolic and oxidative stress parameters in type 2 diabetes

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Abstract:

This study aims to demonstrate the effects of metformin and insulin on metabolic disorders and markers of oxidative stress in Algerian men with type 2 diabetes (T2D), and show the importance of dietary diversity on the impact of these two treatments, in order to recommend the best treatment, which can minimize the complications of diabetes. The study included 120 men recruited from Sabra and 120 men recruited from Maghnia, Tlemcen (Algeria). Each group is divided into four groups (30 healthy control, 30 T2D without treatment, 30 T2D with metformin, and 30 T2D with insulin). A 14-item dietary questionnaire was applied to each individual to define adherence to the Mediterranean diet. Blood samples were collected for the determination of biochemical parameters (glucose, triglycerides, cholesterol, high and low-density lipoprotein cholesterol) and oxidative markers (superoxide anion, nitric oxide, malondialdehyde, carbonyl proteins, oxygen radical absorbance capacity, vitamin C, catalase, glutathione). Our results show that type 2 diabetics from Maghnia have the best adherence score to the Midetanean diet and a diversified diet, richer in fruits, vegetables, fish, and olive oil than those from Sabra. Furthermore, our results show that insulin reduces lipid parameters more than metformin. In addition, the oxidant/antioxidant status became normal in patients treated with metformin especially in patients from Maghnia compared to diabetics from Sabra. The combination of metformin, which reverses the redox changes associated with diabetes and insulin, which improves all lipid profiles, should be prescribed in type 2 diabetes patients with high oxidative stress and hypertriglyceridemia. Moreover, the adaptation of a Mediterranean diet in Maghnia men has provided good control of type 2 diabetes. These results demonstrate the beneficial impact of dietary diversity on the treatment effect in redressing metabolic and oxidative stress disorders in T2D.

Key word: Type 2 diabetes, Diversity, Mediterranean diet, Metformin, Insulin, Oxidative stress.

ملخص:

تهدف هذه الدراسة إلى إظهار تأثير الميتفورمين والأنسولين على الاضطرابات الأيضية وعلامات الإجهاد التأكسدي لدى الرجال الجزائريين المصابين بمرض السكري (T2D) من النوع الثاني وإظهار أهمية التنوع الغذائي على تأثير هذين العلاجين ، من أجل التوصية بأفضل علاج. ، والتي يمكن أن تقلل من مضاعفات (T2D). المرضى والأساليب: أجرينا هذه الدراسة على 120 رجلاً تم تجنيدهم من صبرة و 120 رجلاً تم تجنيدهم من مغنية ولاية تلمسان (الجزائر). تنقسم كل مجموعة إلى أربع مجموعات (30 مجموعة الرجال الاصحاء ، 30 T2D بدون علاج ، 30 T2Dمع ميتفورمين, 30 T2D مع الأنسولين). تم تطبيق استبيان غذائي مكون من 14 عنصرًا على كل فرد لتحديد مدى الالتزام بحمية البحر الأبيض المتوسط. يتم جمع عينات الدم لتحديد المعلمات الكيميائية الحيوية (الجلوكوز ، الدهون الثلاثية ، والكوليسترول ، وكوليسترول البروتين الدهني عالي ومنخفض الكثافة) وعلامات التأكسد (الأنيون الفائق ، وأكسيد النيتريك ، ومالونديالديهيد ، وبروتينات الكربونيل ، وسعة امتصاص جذور الأكسجين ، وفيتامين ج ، والكتلاز الجلوتاثيون). تظهر نتائجنا أن مرضى السكري من النوع 2 من مغنية حصلوا على أفضل درجات الالتزام بالنظام الغذائي المتوسطي واتباع نظام غذائي متنوع ، وغني بالفواكه والخضروات والأسماك وزيت الزيتون أكثر من أولئك الذين ينتمون إلى صبرة. علاوة على ذلك ، تظهر نتائجنا أن الأنسولين يقلل من عوامل الدهون أكثر من الميتفورمين. بالإضافة إلى ذلك ، أصبحت حالة الأكسدة / مضادات الأكسدة طبيعية في المرضى الذين عولجوا بالميتفورمين وخاصة في مرضى مغنية مقارنة بمرضى السكر من صبرة. الخلاصة: يجب وصف مزيج الميتفورمين ، الذي يعكس تغيرات الأكسدة والاختزال المرتبطة بمرض السكري ، والأنسولين ، الذي يحسن جميع ملامح الدهون ، لمرضى السكري من النوع 2 الذين يعانون من الإجهاد التأكسدي العالي وفرط ثلاثي غليسريد الدم. بالاضافة الى ذلك ، فإن اتباع حمية البحر الأبيض المتوسط لدى رجال مغنية قد وفرلهم تحكمًا جيدًا في مرض السكري من النوع 2. كما وضحت هذه النتائج ايضا التأثير المفيد للتنوع الغذائي على تأثير العلاج في تصحيح اضطرابات الإجهاد التأكسدي والتمثيل الغذائي في مرض السكري من النوع 2.

الكلمات المفتاحية: داء السكري من النوع 2 ، النظام الغذائي المتنوع ، حمية البحر الأبيض المتوسط ، الميتفورمين ، الأنسولين ، الإجهاد التأكسدي

Introduction

Diabetics have a variety of lipid abnormalities, which has a significant influence on the occurrence of cardiovascular morbidity and death. Several studies suggest that glucose variability and plasma lipid variability may play a role in the development of diabetes complications (Ceriello and Prattichizzo, 2021). Quantitative and qualitative lipid abnormalities are the most common. Hypertriglyceridemia and low high-density lipoprotein (HDL) cholesterol are quantitative abnormalities, whereas qualitative abnormalities include large proportions of very-low-density lipoprotein (VLDL), enrichment of low-density lipoprotein (LDL) and HDL in triglycerides, LDL oxidation, and glycation of apolipoproteins (Duvillard et al., 2003). Several studies have shown the impact of the Mediterranean diet on these factors (Richard et al., 2013). Adherence to the Mediterranean diet improves insulin resistance and atherogenic dyslipidemia (Bekkouche L et al., 2013).

Protein glycation (PG), also known as non-enzymatic glycosylation, is another cause of hyperglycemia (Heidland et al., 2001; Newsholme et al., 2016; Park et Jae, 2012). Glycation effects circulating proteins. An oxidation process, defined as an unfavorable balance between oxygen free radicals (FR) and antioxidant systems profit first (Ndrepepa G, 2019; Jakubczyk et al., 2020; Vaidya et al., 2021; Rani et al., 2016), might be associated with PG.

Reactive oxygen species (ROS) are chemical species atoms produced from oxygen. A single electron in an FR provides its reactivity, which can cause damage to several molecules (Chainy and Sahoo, 2020; Kumari et al., 2019; Pisoschi et al., 2021; Kancheva et al., 2021). In T2D, however, oxydative stress (OS) is a facilitator of insulin resistance (IR) and its development of glucose intolerance, as well as the pathological installation (Byrne et al., 2021). Cell damage occurs with reduced pancreatic ß-cell activity in severe OS, which is caused by inadequate antioxidant enzyme expression (Pomatto and Davies, 2018). In diabetes mellitus (Merzouk et al., 2004), the oxygen radical absorbance capacity (ORAC) has been discovered to be a useful indicator of OS. Specific antioxidant concentrations in diabetes patients' plasma and erythrocytes have been found to be lower in clinical investigations (Alfonso-Muñoz et al., 2021). The scavenging activities of superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), and ROS were all reduced in T2D individuals (He et al., 2021).

Otherwise, compounds that react with malondialdehyde / thiobarbituric acid, conjugated dienes, lipid hydroperoxides, and isoprostanes (Gaschler and Stockwell, 2017; Van der, 2016) are the main indicators of lipid peroxidation (LP). The production of protein carbonyls and nitrotyrosine groups (Wang et al., 2015) are the two primary biological indicators of protein oxidation. Several investigations have found that T2D plasma contains significant amounts of carbonyls and Advanced Glycation End Products (AGEs), as well as elevated concentrations of LP products (Kattoor et al., 2017).

The first step in the treatment of T2D consists of following hygienic rules. In this context, the Mediterranean diet (MD) is recognized as one of the healthiest dietary patterns in the world (Dinu et al., 2018). Recent findings showed that better adherence to the MD is associated with a reduced incidence of obesity, type 2 diabetes, hypertension, cardiovascular diseases, cancer, and a decrease in biomarkers related to inflammation (San-Cristobal et al., 2017). This diet is diversified and based on a limited intake of saturated fats. Red meat is practically excluded, replaced by fish, dairy products (yogurts, cheese), and lots of fresh or dried fruit and vegetables, all seasoned or cooked with olive oil. The MD health benefits are mainly attributed to its food items known for their anti-inflammatory and antioxidant properties capable to modulate gene and protein expression (Hosseini-Esfahani et al., 2017). When these rules are not sufficient to maintain normal blood sugar levels, medication is necessary. Metformin is frequently the first glucose-lowering therapy in T2D due to its impact on insulin resistance (IR) (Madsen et al., 2019). Metformin improves IR via lowering hepatic glucose output, lowering fasting glycemia, increasing glucose absorption in peripheral tissues, and decreasing hepatic glucose output (Mosenzon et al., 2021; Chun-Yu et al., 2021), and lowers blood sugar levels by inhibiting the synthesis of glucose in the liver (Natali and Ferrannini, 2019). Furthermore, a number of studies have shown that metformin has a positive effect on body mass index (BMI) and body mass composition by reducing fat mass. Hyperinsulinemia, lipid parameters, arterial hypertension, and endothelial dysfunction are also reduced (Madsen et al., 2019). Metformin has been shown to play a function in delaying the progression of impaired glucose tolerance (IGT) to T2D and to improve blood lipid profiles (Zilov et al., 2019). In addition, insulin therapy is eventually required in most T2D patients to maintain satisfactory glycemic control (Inzucchi et al., 2012), even though this intervention has not been shown to lessen the risk of cardiovascular disease (Bakh et al., 2017 ; Hemmingsen et al., 2011 ; Baumgard et al., 2016). Despite a considerable rise in total and LDL cholesterol levels and body weight, some trials found a decrease in cardiovascular risk over the first 18 months of therapy with insulin alone (St EO, 2021). Fasting plasma glucose and glycated hemoglobin (HbA1c) levels were lower in insulin-treated patients, while weight rose (St EO, 2021). The effects of these two treatments differ according to the Mediterranean diet adherence score of the study population. The goal of this study is to compare the effects of metformin and insulin on metabolic disorders and OS markers in Algerian men with T2D in order to determine the best treatment option for reducing diabetes complications and whether or not a combination of metformin and insulin should be recommended. In addition, our objective is to show the relationship between the Midetaraneen diet adherence score and the effect of metformin and insulin in type 2 diabetic populations of Sabra and Meghnia and to demonstrate the necessity of adopting a diversified diet rich in antioxidants to improve the impact of antidiabetic treatments.

Patients and Methods

Participants:

Between January 2018 and June 2019, 120 men were recruited from the diabetic clinic of Sabra Tlemcen (Algeria) and another 120 men from the diabetic clinic of Maghnia Tlemcen (Algeria). Based on the following criteria: non-obese (BMI< 30 kg/m^2), age range between 50 and 60 years, not taking any medication, and not having a chronic disease.

The subjects were divided into four groups: group I consisted of control health, group II consisted of control diabetic men without treatment, group III consisted of type II diabetic men treated with metformin, and group IV consisted of type II diabetic treated with insulin.

All men were also non-smokers. None of the subject's history of high blood pressure (HTA), liver or renal diseases, or a history of cardiovascular diseases.

Information concerning age, BMI, duration of treatment, and blood pressure were collected by questionnaire.

The diabetic patient must have a varied and balanced diet like the Mediterranean diet. In order to define the adherence to the MD diet, each of the participants completed a 14-item Mediterranean diet adherence screener in a face-to-face interview. The questionnaire was applied by us and physicians. The simple 14-item questionnaire was previously developed and used to assess the association between adherence to the MD and the prevalence of metabolic diseases in adults (Echeverría and McGee, 2017; Bakaloudi et al., 2021). The 14-items comprised the olive oil utilization for baking and salads, a large number of fruits, vegetables, cereals, nuts and seeds consumption, low red meat , hamburger, or meat products (ham, sausage, etc.) eating, high fish consumption, low butter, margarine, or cream utilization, and low sweet or carbonated beverages, and cakes, cookies, biscuits. The score of adherence was calculated and a score over 7 indicated a great adherence to the MD.

Participation in this study was voluntary and all subjects gave their written, informed consent. The study was approved by the ethical committee of the Tlemcen-University Hospital.

Blood collection:

Fasting venous blood samples were collected in two tubes; EDTA tubes and dry tubes were centrifuged. Serum was separated for glucose, and lipid parameters. Plasma was separated for oxidant/ antioxidant determinations. Superoxide anion and vitamin C were measured in fresh plasma samples

The remaining erythrocytes were washed and hemolyzed by adding cold distilled water (1/4), and the cell debris was removed by centrifugation (2000g for 15 min). The hemolysates were assayed for antioxidant enzyme activities and GSH contents.

***Biochemical analysis*:**

Serum glucose, triglycerides, and cholesterol contents were determined by enzymatic methods (Kits Sigma Chemical Company, St Louis, MO, USA).

***Lipoprotein isolation*:**

Total lipoproteins were isolated from plasma by precipitation according to Burstein et al (1989). Lipoprotein (LDL and HDL) triglyceride and total cholesterol contents were determined by enzymatic methods (Kits from Sigma).

Oxidant/antioxidant marker determination:

Scavenging capacity of plasma: The oxygen radical absorbance capacity of plasma (ORAC) employs the oxidative loss of the intrinsic fluorescence of allophycocyanin (APC) (Merzouk et al., 2004).

Determination of plasma level vitamin C:

Plasma vitamin C levels were determined in plasma by using the method of Roe and Kuether (Roe and Kuether, 1943).

Determinations of erythrocyte antioxidant enzyme activities:

Determination of Erythrocyte catalase (EC 1.11.1.6) activity was measured by spectrophotometric analysis of the rate of hydrogen peroxide decomposition at 240 nm (Sigma Aldrich kit). Erythrocyte-reduced glutathione (GSH) levels were assayed by a colorimetric method, according to a Sigma Aldrich kit (Saint Louis, USA).

Determination of superoxide anion:

The spectrophotometric determination of the $O_2^{\bullet-}$ was based on the reduction of nitroblue tetrazolium (NBT) in the presence of superoxide anion ($O_2^{\bullet-}$), a chromophore that absorbs at 550 nm (Auclaire and Voisin, 1985).

Determination of nitric oxide NO:

Plasma and erythrocyte NO were determined by the method of Guevara et al (1998) after deproteinization using the colorimetric method of Griess.

Determination of malondialdehyde:

Plasma malondialdehyde (MDA) levels were determined by the reaction of MDA with thiobarbituric acid (Sigma Aldrich kit; St. Louis, MO, USA).

***Determination of Carbonyl Proteins*:**

Plasma carbonyl proteins (CP) were determined by the derivatization of protein carbonyl groups with 2, 4-dinitrophenylhydrazine leading to the formation of stable dinitrophenyl hydrazone adducts (Sigma Aldrich kit).

Statistical analysis:

The results are presented as means and of men are performed by ANOVA one factor. This analysis is completed by Tukey's test to locate the source of the significant difference. The Means indicated by different superscript letters (a, b, c, d) are significantly different (p <0.05). All tests were performed using STATISTICA 4.1 program (StatSoft, Tulsa, OK). Standard deviations (SD). A priori power analysis was performed to determine the sample size, using a power and sample size calculator (Statistical solutions, Sigma). The results were tested for normal distribution using the Shapiro-Wilk test. The comparison of means between the four groups is performed by ANOVA one factor.

Results

Clinical and Biochemical Parameters: Table 1 shows that BMI was significantly higher in T2D without treatment and T2D treated with insulin and metformin compared with control subjects in Sabra and Maghnia men. BMI did not differ significantly between T2D with metformin and T2D with insulin (p>0.05). BMI of Maghnia subjects was reduced compared to Sabra subjects. No significant difference was found among the four groups for Blood pressure (systolic, SBP, and diastolic, DBP). Additionally, significant differences were found between T2D with metformin, and T2D with insulin compared to control subjects and T2D without treatment for plasma glucose levels Table 2. T2D without treatment in Sabra and Maghnia have the highest glucose concentrations (ANOVA, p<0.001). The glucose level is reduced in treated diabetic patients from Maghnia compared to Sabra. The score of adherence to the Mediterranean diet calculated in Maghnia subjects was over 7 compared to 5 in Sabra's subjects. This difference is related to the dietary and cultural diversity of each region and socio-economic status. Maghnia men's diet was diversified and rich in vegetables, fruit, fish, and olive oil.

Lipid and Lipoprotein Levels: The highest values of total cholesterol were observed in T2D without treatment and T2D with metformin patients compared with their control and T2D with the insulin treatment group Table 2 (p<0.001, p>0.05, p<0.01). Maghnia men showed a decrease in plasma total cholesterol levels compared to Sabra men. Triglycerides levels were reduced in treated diabetic and control compared to untreated. Metformin and insuline reduce triglyceride concentration in T2D men. A greater reduction was observed with insulin treatment in Maghnia men (p<0.05). Lipoprotein concentrations were markedly different among the four groups studied (Table2). LDL-C amounts were significantly higher in T2D without treatment compared to T2D treated with metformin or insulin compared to control.

Table 1 : Characteristics of the study population

Characteristic	control	T2D without treatment	T2D with metformin	T2D with insulin	ANOVA
Sabra					
Number (n)	30	30	30	30	
Age (years)	53 ± 3	52 ± 2	54 ± 3	56 ± 4	
BMI (Kg/m²)	22.49±1.23	27.06±1.89^{a}	25.83±1.85^{b}	25.94±1.60^{b}	0.0001
Duration of treatment	-	-	7±3	5±1	
SBP (mm Hg)	124±5.25	130±8.25	127±7.11	120.43±5.26	0.120
DBP (mm Hg)	76.53±4.55	84.35±5.79	80.43±5.38	86.32±5.12	0.110
Maghnia					
Number (n)	30	30	30	30	
Age (years)	55 ± 2	51 ± 4	53 ± 2	52 ± 4	
BMI (Kg/m²)	20.29±1.32^{c}	25.16±1.56^{a}	23.33±1.58^{b}	23.45±1.50^{b}	0.002
Duration of treatment	-	-	7±3	5±1	
SBP (mm Hg)	126±4.27	132±8.38	128±5.31	122.25±3.32	0.130
DBP (mm Hg)	70.55±5.25	82.23±4.72	79.52±6.23	78.25±6.12	0.140

Values are means ± SD. BMI, body mass index ; DBP, diastolic blood pressure ; SBP, systolic blood pressure. Statistical comparison between the four groups (Control, T2D without treatment, T2D treated with metformin, and T2D treated with insulin) was performed by a one-way ANOVA test, followed by a Tukey post hoc test. Values for each parameter with different superscripts (a,b,c,d) are significantly different for p < 0.05, as determined by one-way ANOVA and the least significance test. p values for the ANOVA test.

LDL-C levels were significantly higher in T2D with metformin compared to T2D with insulin. LDL-C concentrations are more reduced in Maghnia subjects compared to Sabra men. HDL-C concentrations were significantly higher in T2D with metformin and T2D with insulin compared to control subjects and T2D without treatment. No significant difference betweenT2D with metformin and T2D with insulin for HDL-C. The report LDL- C/HDL-C levels were significantly lower in T2D with metformin and T2D with insulin than controls group and T2D without treatment (p<0.001), but the differences did not reach statistical significance between T2D with metformin and T2D with insulin (p> 0.05).

Table 02: Biochemical parameters of the study population.

Parameter	control	T2D without treatment	T2D with metformin	T2D with insulin	ANOVA
Sabra					
Glucose (mmol/L)	4.98±0.27^d	7.27±0.49^a	6.52±0.54^b	5.96±0.24^c	0.0001
TG (mmol/L)	1.45±0.09^c	1.85±0.22^a	1.66±0.15^b	1.56±0.17^b	0.0400
TC (mmol/L)	4.67±0.31^b	5.53±0.22^a	5.45±0.50^a	4.72±0.46^b	0.0001
LDL-C (mmol/L)	2.72±0.44^b	3.72±0.20^a	2.28±0.34^c	1.85±0.45^d	0.0001
HDL-C (mmol/L)	1.58±0.25^b	1.12±0.10^c	2.32±0.26^a	2.44±0.24^a	0.0001
LDL-C/HDL-C	1.81±0.48^b	3.37±0.39^a	0.99±0.23^c	0.81±0.12^c	0.0001
Maghnia					
Glucose (mmol/L)	4.55±0.17^d	7.57±0.52^a	5.92±0.45^b	4.99±0.34^c	0.0001
TG (mmol/L)	0.83±0.20^c	1.52±0.12^a	1.32±0.45^b	1.26±0.27^b	0.0400
TC (mmol/L)	4.51±0.35^b	4.92±0.24^a	4.72±0.60^a	4.53±0.50^b	0.0001
LDL-C (mmol/L)	3.23±0.54^b	3.56±0.25^a	2.78±0.26^c	2.05±0.35^d	0.0002
HDL-C (mmol/L)	1.44±0.15^b	0.93±0.19^c	2.29±0.36^a	2.65±0.29^a	0.0001
LDL-C/HDL-C	2.16±0.52^b	3.75±0.26^a	1.29±0.35^c	0.99±0.25^c	0.0004

Oxidative Stress Biomarkers :

***Oxidant Status*:**

For erythrocyte O2•-, the statistical study demonstrated a significant difference among the four groups. The levels of O2•- were significantly higher in T2D with metformin and T2D with insulin compared to T2D without treatment and controls (p<0.001) Table 3. The highest level of O2•- was observed in T2D with insulin. Compared to Sabra subjects, men from Maghnia have reduced erythrocyte O2•- values. A significant difference between T2D with metformin and T2D with insulin was found for erythrocyte NO. The highest NO levels were observed in T2D treated with insulin compared to T2D without treatment and controls. However, no significant difference in NO was observed between T2D with Metformin and T2D without treatment Table 3. The reduced levels of erythrocyte NO were recorded in men from Maghnia. Erythrocyte MDA levels were higher in all diabetic patients, treated with metformin or insulin and not treated compared with control men, but T2D without treatment men's values were the highest (ANOVA p<0.001 and p <0.001, respectively) Table 3.

Table 3: Oxidant intracellular markers in the study population.

Parameter	control	T2D without treatment	T2D with metformin	T2D with insulin	ANOVA
Sabra					
O2 (µmol/L)	50.91±2.01^d	56.17±2.16^c	76.27±5.53^b	84.10±3.62^a	0.0001
NO (µmolL)	38.16±3.06^b	34.82±4.41^c	35.42±2.92^c	48.93±6.05^a	0.0001
MDA (µmol/L Lyat)	2.11±0.27^c	6.17±0.26 ^{a}	3.35±0.45^b	4.02±0.33^b	0.0001
CP (nmol/mg protein)	2.7±0.33^c	6.15±0.29^a	3.98±0.40^b	3.82±0.38^b	0.0006
Maghnia					
O2 (µmol/L)	45.32±1.02^d	51.36±3.18^c	60.12±2.45^b	65.21±4.20^a	0.0001
NO (µmolL)	32.30±3.20^b	30.23±2.64^c	31.21±1.25^c	39.65±4.56^a	0.0004
MDA (µmol/L Lyat)	1.02±0.21^c	5.18±0.25 ^{a}	2.15±0.15^b	2.56±0.55^b	0.0006
CP (nmol/mg protein)	2.30±0.045^c	5.05±0.32^a	2.96±0.12^b	3.15±0.29^b	0.0006

On the other hand, erythrocyte MDA concentrations are reduced in men from Maghnia compared to those from Sabra. Similarly, erythrocyte carbonyls protein level was enhanced in T2D without treatment and T2D treated with metformin or with insulin compared to control cases ($p<0.0006$) and significantly decreased with the two treatments compared to T2D without treatment in both regions. The most important decrease was observed in the Maghnia groups.

***Antioxidant Status*:**

Oxidative stress biomarkers Plasma total antioxidant status (ORAC) was significantly lower in T2D without treatment compared to controls (Table 4). The values tended to increase in the treated group compared to the untreated remarkably in the Maghnia population, but there was no significant difference between the metformin and insulin treated group in the two regions. Vit C levels were significantly lower in T2D without treatment compared to the control and treated groups, but it was significantly higher in T2D treated with metformin compared to T2D treated with insulin ($p<0.001$) Table 4.

Table 4: Antioxidant markers in the study population

Parameter	control	T2D without treatment	T2D with metformin	T2D with insulin	ANOVA
Sabra					
ORAC (UI)	4.54±0.26^{a}	1.58±0.25^{c}	2.55±0.27^{b}	2.35±0.38^{b}	0.0001
Vit C (µmol/L)	47.48±2.25^{a}	27.92±1.28^{c}	45.88±5.87^{a}	35.36±3.12^{b}	0.0001
Catalase (UI/min/ml)	88.73±2.57^{a}	49.21±1.62^{c}	69.60±5.92^{b}	67.58±4.86^{b}	0.0001
GSH (mmol/L)	1.55±0.06^{a}	0.59±0.14^{b}	1.51±0.08^{a}	1.54±0.06^{a}	0.0010
Maghnia					
ORAC (UI)	5.50±0.16^{a}	2.50±0.12^{c}	3.98±0.78^{b}	4.56±0.45^{b}	0.0001
Vit C (µmol/L)	60.54±3.54^{a}	40.52±2.20^{c}	50.54±6.20^{a}	46.32±2.25^{b}	0.0002
Catalase (UI/min/ml)	95.32±4.52^{a}	68.23±2.56^{c}	79.32±3.35^{b}	85.32±6.98^{b}	0.0004
GSH (mmol/L)	2.05±0.09^{a}	0.89±0.16^{b}	1.89±0.05^{a}	1.95±0.07^{a}	0.0010

The highest values were observed among Maghnia men with control and T2D with metformin. Erythrocyte catalase activities were markedly different among the group studied, it was significantly lower in T2D without treatment and T2D treated with metformin and insulin compared to the control subject Table 4. Diabetes medication induced a rise in catalase activities but dis not reach control values after treatment with insulin and metformin. The improvement of catalase activity is considerably more observed in the studied population of Maghnia. Erythrocyte GSH values were significantly lower in T2D without treatment compared to control subjects and T2D treated with metformin and T2D with insulin ($p<0.001$) Table 4. Erythrocyte GSH values improved remarkably in treated T2D from Maghnia compared to Sabra. However, no significant difference in erythrocyte GSH activities was observed between controls cases and in both T2D-treated patients.

Discussion

The traditional Mediterranean diet being rich in olive oil, vegetables, fruits, nuts, cereals, legumes, and fish, but relatively low in meat and dairy products presents a typical model of a healthy and varied diet. Several studies have shown that the Mediterranean diet plays a preventive role against cardiovascular diseases (CVD) (Martinez-Gonzalez et al., 2004 ; Estruch et al., 2013). In our study, the assessment of the Mediterranean diet adherence score allows us to evaluate the association between the Mediterranean diet, the dietary biodiversity and the prevalence of metabolic diseases such as diabetes and the correction of the resulting metabolic and oxidative disorders (Bekkouche et al., 2013). The cultural, social, and environmental diversities that exist between the two groups studied in Maghnia and Sabra influence their eating habits. The latter has a definite impact on gene expression and can therefore act to prevent the development of chronic diseases such as diabetes and its complications, which explains this diversity between the men recruited in Maghnia and Sabra. Indeed, a study has shown the impact of precarity on the diet of diabetics (Vannereau et al., 2014), which reveals that a low-income person who eats little fruit and vegetables, meat, and eggs but consumes more alcohol and soft drinks has a greater risk of complications with diabetes.

Diabetes can induce multiple changes in lipids, lipoproteins, and oxidant/antioxidant status. The results obtained in the present study are consistent with previous findings (Ting et al., 2021; Azarpazhooh et al., 2021 ; Hirano et al., 2021). Indeed, our results indicate that metformin and insulin are efficacious substances in the treatment of diabetes as there is a significant decrease in plasma glucose levels in T2D patients treated with metformin and insulin compared to untreated diabetics from Maghnia and Sabra. The effect of metformin on blood glucose levels is attributed to a decrease in hepatic glucose production and an increase in glucose transport in muscle cells (Zhou et al., 2001). The high adherence score to the Mediterranean diet (9/14) observed in Maghnia subjects compared to Sabra subjects can explain the significant improvement in their blood glucose levels.

Several studies (Ceriello et al., 2014, Estruch et al., 2006: Oh et al., 2010) have shown the protective effect of olive oil against insulin resistance, the main step in the development of T2D (Schlienger&Monnier, 2020). The consumption of olive oil, rich in monounsaturated fatty acids (MUFA), is beneficial for diabetics as it improves insulin sensitivity and lipid profile (Santangelo et al, 2016).

This type of diet also improves markers of glucose homeostasis (36% fasting blood glucose and 22% insulin levels) (Demosthenes et al., 2007; Elhayany et al., 2011). In addition, a study of postmenopausal women with T2D showed that adaptation to the diversified Mediterranean diet improved serum glucose control (Toobert et al., 2003) and reduce glycated hemoglobin (-0.4% HbA1c) in diabetics consuming a low glycemic index diet (Brand et al., 2003).

Glycaemic control is not the only objective for diabetics, improving the lipid profile, weight, and blood pressure are factors to be taken into consideration. Several studies have shown the impact of the MD on these factors (Richard et al., 2013 ; Panagiotakos et al., 2004, Psaltopoulou et al., 2004).

In this study, lipid levels are altered, and lipoprotein content is significantly modified in diabetic patients compared to controls. Diabetic patients had elevated plasma triglycerides, cholesterol and LDL-C levels, but low HDL-C levels. These results are consistent with previous studies (Shah, 2019 ; Poznyak et al., 2020 ; Summerhill et al., 2019 ; Taleb, 2016). In addition, a high atherogenicity ratio (LDL-C/HDL-C) was observed in patients with T2D. These abnormalities could be attributed to hyperglycemia, insulin resistance, lipase, and cholesterol ester transfer protein (CETP) activities (Taleb, 2016 ; Vergès, 2009 ; Stankova et al., 2019). In this study, men from Maghnia have lower plasma triglyceride levels than men from Sabra, which is related to the adoption of an MD. According to Mosbah et al. ,2018, adherence to the MD seems to protect against the occurrence of overweight and decreases triglycerides and improves glycaemic control in type 2 diabetics (Bakaloudi et al., 2021).

Our findings showed that patients with T2D had high levels of intracellular pro-oxidant markers (O2--, CP and MDA) and low concentrations of antioxidants (vitamin C, catalase and GSH). These results are consistent with those reported in previous studies (Roma and Jean-Christophe, 2020). ORAC values were significantly lower in untreated T2D patients compared to non-diabetic controls. In diabetes, several mechanisms seem to be involved in the genesis of oxidative stress. Indeed, the auto-oxidation of glucose in the presence of iron leads to the generation of reactive oxygen species (ROS) (Gerber and Rutter, 2017 ; Yan, 2014), and leads to protein glycation, overproduction of superoxide radicals at the mitochondrial and NADPH oxidase levels (Pitocco et al., 2010 ; Xiao-Bin et al., 2016 ; Ola et al.,2006 ; Rosca et al., 2012 ; Kulaksızoglu and Karalezli, 2016 ; Oguntibeju, 2019). Reduced levels of (NO) were observed in patients (T2D), which is in agreement with the results reported in a previous study (Pitocco et al., 2010 ; Oleson and John , 2018). Overproduction of (NO) is associated with various inflammatory conditions, including diabetes (Afonso et al., 2007 ; Velagic et al., 2020 ; Al-nimer et al., 2010 ; Luvuno et al., 2021 ; Sanz-Cameno et al., 2002). In this work, subjects from Sabra who adopt a classic diet, have elevated concentrations of oxidants compared to Maghnia subjects. Adherence to the MD also reveals an effect attested by an improvement in oxidative status by a significant decrease in plasma, erythrocyte, and platelet levels of hydroperoxides and carbonyls (Bekkouche *et al*, 2013).

The results of this study show a decrease in plasma triglyceride and LDL-C levels and an increase in HDL-C levels. Metformin and insulin therapies normalize lipid and lipoprotein levels in T2D. However, total cholesterol concentrations can only be normalized with insulin. In another study, it was indicated that metformin may play a major role in reducing blood cholesterol (Van et al., 2018; Vergès, 2001). Low glycaemic index foods improve the lipid profile of diabetics by lowering LDL cholesterol levels and reducing weight (Heilbronn et al., 2002).

In this study, it is shown that T2D patients prescribed metformin showed a reduction in LDL-cholesterol, in contrast to untreated T2D patients, which is in agreement with the results reported in previous studies (Shokrpour et al., 2019 ; Geerling et al., 2014). Furthermore, metformin and insulin also contributed to a reduction in the atherogenicity ratio (LDL-C/HDL-C) in (T2D) patients, unlike untreated patients. The greatest reduction in this ratio was observed in patients treated with insulin. This contributes to the reduction of major cardiovascular risk factors. The Maghnia subjects consumed a diversified diet rich in olive oil, fish and low glycaemic index carbohydrates that followed the Mediterranean model, which improved their lipid profile. These results are consistent with previous studies (Heilbronn et al., 2002).

Several studies have shown that high triglyceride and blood sugar levels contribute to higher (OS) (Yaribeygi et al., 2020). The results of the present investigation confirm a strong association between plasma glucose concentrations and (OS) parameters. Oxidative stress in diabetes is generally caused by a decrease in the antioxidant defense system and an increase in ROS production due to hyperglycemia (Zhuang et al., 2014). In addition, chronic hyperglycemia induces increased protein oxidation in patients with T2D (Alhagh et al., 2021).

The results of this study indicate that insulin-treated T2D patients have higher levels of certain pro-oxidant markers (NO, O2--) compared to metformin-treated T2D patients. In addition, low levels of MDA and PC were observed in T2D patients treated with both insulin and metformin, in contrast to untreated diabetic patients, which is in good agreement with those reported in previous studies (Grindel et al., 2016).

The better glycaemic control observed in T2D patients treated with insulin and metformin can explain the decrease in radical production and lipid peroxidation. In our study, markers of protein oxidation, such as carbonyls, showed a significant decrease in protein oxidation in diabetic patients treated with insulin and metformin, in contrast to untreated patients. This decrease is attributed to low blood glucose levels in diabetic patients treated with insulin and metformin. This is consistent with the results of a study, which indicates that appropriate glycaemic control decreases plasma carbonyl levels in patients with T2D (Alhagh et al., 2021). Moreover, the results of the present study indicate a significant decrease in erythrocyte, superoxide anion and nitric oxide (NO) levels in metformin-treated T2D patients compared to insulin-treated patients. Thus, the decrease in (NO) production in diabetic patients is the cause of impaired endothelial vasodilation (Santilli et al., 2004). On the other hand, (NO) levels are higher in insulin-treated T2D patients as compared to those treated with metformin and those not treated. This is probably due to improved glycemic control, which induces the reduction in the (OS).

The present study is an attempt to measure some markers of antioxidant defense, vitamin C level, catalase activity, (OS) biomarkers, total plasma antioxidant (ORAC), and glutathione concentration. Levels of antioxidants, vitamin C, catalase and GSH were found to be higher after treatment and remarkably in Maghnia subjects with a high MD adherence score. Adherence to the MD reveals an antioxidant effect evidenced by an improvement in oxidative status through an increase in antiradical enzymes (Bekkouche et al.,2013). These results demonstrate the correlation between the dietary biodiversity observed in the MD and the improvement of antioxidant defense in the treated Maghnia population. ORAC was significantly lower in untreated T2D patients than in control patients but remained unchanged between patients treated with metformin and insulin.

The results of this study indicate higher plasma vitamin C levels in metformin-treated T2D patients as compared to insulin-treated patients. The increase in vitamin C levels may be explained by the increased concentration of glutathione, which is an essential factor for the enzymatic regeneration of ascorbic acid from dehydroascorbate (Cammisotto et al., 2021). Among the men of Maghnia, the increase in plasma levels of vitamin C may be related to the nutritional intake of this vitamin through the consumption of a diversified diet rich in fruits and vegetables, the main characteristic of the MD.

The results obtained revealed a significant increase in erythrocyte catalase activity in treated diabetic patients compared to untreated subjects. These results are consistent with those of several other authors who have reported a decrease in erythrocyte catalase activity in diabetic patients (Merzouk et al., 2004).

Glutathione (GSH) is the main soluble antioxidant in cells (Orhan et al., 2003 ; Xepapadaki et al., 2020). The results of this study indicated a significant difference in erythrocyte GSH levels between treated and untreated diabetic patients, in favor of an improved antioxidant defense. Our results highlight the beneficial effects of metformin and insulin on the lipid and lipoprotein profiles of patients with T2D. Favorable changes in lipid and lipoprotein parameters were observed in T2D patients treated with insulin, in addition, there was a decrease in plasma total LDL cholesterol and triglyceride levels and an increase in HDL-C levels. These treatments normalize lipid and lipoprotein levels in diabetic patients. The MD, due to its diversity and richness in antioxidants, decreases the oxidation of LDL, a marker of oxidative stress and a major factor in the development of atherosclerosis (Mitjavila et al., 2012 ; Holvoet et al., 2001, Meisinger et al., 2005). Olive oil also has a high phenolic antioxidant phenolic power (Owen et al., 2000). The consumption of nuts, a feature of the MD, is a source of phytochemical antioxidant nutrients (Kris-Etherton et al., 2001 ; Mirmiran et al., 2018 ; Abete et al., 2008). In addition, the richness of fish provides a good supply of omega-3. The latter reduces serum triglyceride and lipid peroxide levels (Kesavulu et al., 2002). Results of previous studies can explain the notable improvement in the antioxidant/oxidant balance in Maghnia subjects with a higher MD adherence score compared to Sabra subjects and confirm the relationship between the dietary diversity and the good management of diabetics under treatments.

The present work found that after treatment with metformin and insulin, plasma levels of MDA and carbonylated proteins were significantly decreased, while levels of vitamin C, GSH and catalase activities were remarkably increased. However, the results suggest that insulin reduces total and LDL cholesterol more than metformin; it also increases NO and O-2 production. Our findings confirm the association between MD adherence score and (CVD) prevention in type 2 diabetics. A diversified diet observed in our subjects from Maghnia with a high MD adherence scores allows a strong improvement of lipid profile and oxidative status with metformin and insulin.

Conclusion

In conclusion, the present study suggests that reducing blood glucose levels in T2D subjects reduces ROS generation. Both metformin and insulin show beneficial effects on plasma lipids or markers of oxidative stress in diabetic patients with pre-existing plasma lipid abnormalities and oxidoreductases. In addition, insulin improves the lipid profile and metformin reverses the redox changes associated with diabetes. It can be concluded that the combination of metformin and insulin can help to correct the oxidative status and lipid disorders observed in diabetic patients. In addition, the adaptation of a diversified diet such as the Mediterranean diet also seems very beneficial in the management of type 2 diabetics. MD offers type 2 diabetics an effective antioxidant defense that slows the development of complications. In our study, the diet is well-diversified among the participants in Maghnia compared to Sabra. Which justifies the significant improvement in oxidative stress parameters, recorded in Maghnia patients treated with metformin and insulin compared to those in Sabra. Diversity diet is the most important factor in the management of T2D to achieve good metabolic control.

A change in dietary behavior is necessary to avoid hyperglycemia and excess weight. This can reduce the need for insulin. The key to successful diabetes treatment is regular physical activity, combined with a healthy, varied, and balanced diet to help stabilize blood sugar levels, improve quality of life, and to slow the development of complications associated with T2D. It is therefore important to note that maintaining dietary diversity is a point to be taken into consideration in the management of metabolic diseases, in particular diabetes. However, this diversity will only be accessible with the maintenance of biodiversity in our crops.

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Author contributions

R.H designed the study, carried out the field and laboratory work, performed the statistical analysis, and wrote and edited the manuscript. G.B supervised the study and contributed to the writing of the manuscript. M.H. revised and contributed to the writing of the manuscript. M.S. participated in the statistical analysis

Disclosure statement

No potential conflict of interest was reported by the authors.

References

Abete I, Parra D, Martinez JA. 2008. Energy-restricted diets based on a distinct food selrction affecting the glycemic index induce different weight loss and oxidative response. Clin Nutr; 27 :545-551.

Afonso V, Champy R, Mitrovic D. 2007. Collin P and Lomri A: Reactive oxygen species and superoxide dismutases: role in joint diseases, Jt. Bone Spine. 74: 324-29.

Alfonso-Muñoz EA, Burggraaf-Sánchez de Las RM, Boronat JM, César J, Martín M and Desco C: 2021. Role of Oral Antioxidant Supplementation in the Current Management of Diabetic Retinopathy. International Journal of Molecular Sciences; 22(8): 4020.

Alhagh E, Gorgich C, Parsaie H, Yarmand S, Baharv F and Sarbishegi M 2021. Long-Term administration of metformin ameliorates age-dependent oxidative stress and cognitive function in rats. Behav Brain Res 113343.

Al-nimer MSM, Al-obaidi SAH and Al-dulaimi KS. 2010: Serum nitric oxide and Peroxynitrite levels in adult seropositive rheumatoid arthritis treated with disease modifying antirheumatic drugs: a preliminary report, Turk. J Med Sci; 40-191e197.

Auclaire C and Voisin E. 1985. Nitrobluetetrazolium reduction. In: Greenworld R A, editor. Handbook of Methods for Oxygen Radical Research. Boca Raton: CRC Press, Inc; 123-32.

Azarpazhooh MR, Najafi F, Darbandi M, Kiarasi S, Oduyemi T and Spence JD. 2021 Triglyceride/high-density lipoprotein cholesterol ratio: a clue to metabolic syndrome, insulin resistance, and severe atherosclerosis. Lipids. Doi: 10.1002/lipd.12302.

Bakaloudi DR, Chrysoula L, Kotzakioulafi E, Chourdakis M. 2021 Impact of the Level of Adherence to Mediterranean Diet on the Parameters of Metabolic Syndrome: A Systematic Review and Meta-Analysis of Observational Studies. Nutrients , 13(5), 1514.

Bakh NA, Cortinas AB, Weiss MA, Langer RS, Anderson DG., Gu Z, Dutta S and Strano MS 2017. Glucose-responsive insulin by molecular and physical design. Nat Chem; 9: 937-43.

Baumgard LH, Hausman GJ and Fernandez MVS. 2016. Insulin: pancreatic secretion and adipocyte regulation. Domest Anim Endocrinol 54: 76-84.

Bekkouche Linda et al. 2013 The Mediterranean Diet Adoption Improves Metabolic, Oxidative and Inflammatory Abnormalities in Algerian Metabolic Syndrome Patients. Horm Metab Res; 45: 1-9.

Brand-Miller J, Hayne S, Petocz P, Cola-giuri S. 2003. Low-glycemic index diets in the management of diabetes: a meta-analysis of randomized controlled trials. Diabetes Care,26: 2261-2267.

Burstein M, Fine A, Atger V, Xirbel E and Girard-Globa A. 1989. rapid method for isolation of two purified subfraction of high density lipoproteins by differential dextran sulfatemagnesium choride precipitation. Biochem; 71: 741- 46.

Byrne NJ, Rajasekaran NS, Abel ED and Bugger H. 2021. Therapeutic potential of targeting oxidative stress in diabetic cardiomyopathy. Free Radic Biol Med; 169: 317-42.

Cammisotto V, Nocella C, Bartimoccia S, Sanguigni V, Francomano D, Sciarretta S, Pastori D, Peruzzi M, Cavarretta E, D'Amico A, Castellani V, Frati G, Carnevale R and SMiLe Group. 2021.The Role of Antioxidants Supplementation in Clinical Practice: Focus on Cardiovascular Risk Factors. Antio (Basel); 10(2): 14

Ceriello A, Esposito K, La Sala L, pujadas G et al. 2014. The protective effect of the Mediterranean diet on endothelial resistance to GLP-1 in type 2 diabetes : a preliminary report. Cardiovascular Diabetology ; volume 13, Article number: 140.

Ceriello A and Prattichizzo F 2021. Variability of risk factors and diabetes complications. Cardiovasc Diabetol; 20(1): 7.

Chainy GBN and Sahoo DK. 2020. Hormones and oxidative stress: an overview. Free Radic Res 54(1): 1-26.

Chun-Yu L, Chun-Hsin W, Chung-Yuan H, Tien-Hsing C, Ming-Shyan L, Yu-Sheng L and Yu-Jih S: 2021. Reduced mortality associated with the use of metformin among patients with autoimmune diseases. Front Endocrinol (Lausanne).; 12: 641635.

Demosthenes B. Panagiotakos N. Tzima, Christos P. Chrysohoou C. Zampelas A. Toussoulis D. Christodoulos S. 2007 The Association between Adherence to the Mediterranean Diet and Fasting Indices of Glucose Homoeostasis: The ATTICA Study. Journal of the American College of Nutrition , Vol. 26, No. 1, 32–38.

Dinu *M et al.* 2018. Mediterranean diet and multiple health outcomes: an umbrella review of meta-analyses of observational studies and randomised trials Eur. J. Clin. Nutr.

Duvillard L, Florentin E and Lizard G 2003. Cell Surface Expression of LDL Receptor is decreased in type 2 diabetic patients and is Normalired by insulin Therapy. Diabet Care.; 26: 1540-4.

Echeverría G and. McGee E. 2017. Inverse Associations between a Locally Validated Mediterranean Diet Index, Overweight/Obesity, and Metabolic Syndrome in Chilean Adults. Nutrients , 9(8), 862.

Elhayany A, Lustman A, Abel R, Attal-Singer J, Vinker S. A 2011. Lowcarbohydrate Mediterranean diet improves cardivascular risk factors and diabetes control among overweight patients with type 2 diabetes mellitus : a 1-year prospective randomized intervention study. Diab obes Metab ; 12 :204-209.

Estruch R, Martinez-Gonzalez MA, Corella D, Salas-Salvado J, Ruiz-Gutiérrez V, Covas MI, et al. 2006. Effects of a Mediterranean-style diet on cardiovascular risk factors : a randomized trial. Ann Intern Med, ;145:1-11.

Estruch R, Ros E, Salas-Salvado J, Covas MI, Corella D, Aros F, Gomez-Gracia E, Ruiz-Gutierrez V, Fiol M. 2013. Primary prevention of cardiovascular diesease with a Mediterrnean diet.N Engl J Med ;368 :1279-1290.

Gaschler MM and Stockwell BR. 2017 Lipid peroxidation in cell death. BiochemBiophys Res Commun; 482(3): 419-25.

Geerling JJ, Boon MR and van der Zon GC, van den Berg SA and van den Hoek AM. 2014. Metformin lowers plasma triglycerides by promoting VLDL-triglyceride clearance by brown adipose tissue in mice. Diabetes 63(3): 880-91.

Gerber PA and Rutter GA: 2017. The Role of Oxidative Stress and Hypoxia in Pancreatic Beta-Cell Dysfunction in Diabetes Mellitus. Antioxid Redox Signal 26(10): 501-18.

Grindel A, Guggenberger B, Eichberger L, Pöppelmeyer C, Gschaider M, Tosevska A, Mare G, Briskey D, Brath H and Karl-Heinz Wa. 2016. Oxidative Stress, DNA Damage and DNA Repair in Female Patients with Diabetes Mellitus Type 2.PLoS One.; 11(9): e0162082

Guevara I, Iwanejko J and Dembińska-Kieć A. 1998 Determination of nitrite/nitrate in human biological material by the simple Griess reaction. ClinChim Acta 274: 177-88.

He X, Kuang G, Yi Z, Li S, Zhou S and Ou C. 2021. The role of non-coding RNAs in diabetic nephropathy-related oxidative stress. Front Med (Lausanne). 8: 626423.

Heidland A, Sebekova K and Schinzel R: 2001. Advanced glycationproducts and the progressive course of renal disease. Am J Kidney Dis.; 38: S100-S106.

Heilbronn LK, Noakes M, Clifton PM. 2002. The effect of high- and low-glycemic index energy restricted diets on plasma lipid and glucose profiles in type 2 diabetic subjects with varying glycemic control. J Am Coll Nutr.;21(2):120-7.

Hemmingsen B, Lund SS and Gluud C: 2011. Intensive glycaemic control for patients with type 2 diabetes: systematic review with meta-analysis and trial sequential analysis of randomised clinical trials; BMJ .343:d6898.

Hirano T, Kodera R, Hirashima T, Suzuki N, Aoki E, Hosoya M, Oshima T, Hayashi T, Koba S, Ohta M, Satoh N and Ito Y 2021. Metabolic properties of lowdensity lipoprotein (ldl) triglycerides in patients with type 2 Diabetes, Comparison with Small Dense LDL-Cholesterol. J Atheroscler Thromb 2021. Doi: 10.5551/jat.62789.

Holvoet P, Mertens A, Verhamme P, et al. 2001. Circulating oxidized LDL is a useful marker for identifying patients with coronary artery disease. Arterioscler Thromb Vasc Biol;21:844-848.

Hosseini-Esfahani F *et al. 2017*. Mediterranean Dietary Pattern Adherence Modify the Association between FTO Genetic Variations and Obesity Phenotypes Nutrients (2017).

Inzucchi SE, Bergenstal RM and Buse JB: 2012. Management of hyperglycaemia in type 2 diabetes: a patient-centered approach. Position statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). Diabetologia 2012; 55: 1577– 96.

Jakubczyk K, Dec K, Kałduńska J, Kawczuga D, Kochman J and K Janda: 2020. Reactive oxygen species - sources, functions, oxidative damage. Pol Merkur Lekarski 2020; 48(284): 124-27.

Kancheva VD, Dettori MA, Fabbri D, Alov P, Angelova SE, Slavova-Kazakova AK, Carta P, Menshov VA, Yablonskaya OI, Trofimov AV, Tsakovska I and Sas L. 2021 Natural Chain Breaking Antioxidants and Their Synthetic Analogs as Modulators of Oxidative Stress. Antioxidants (Basel). 10(4): 624.

Kattoor AJ, Pothineni NVK, Palagiri D and Mehta JL. 2017. Oxidative Stress in Atherosclerosis. Curr Atheroscler Rep 19(11): 42.

Kesavulu M.M, Kameswararao B , Apparao CH ,. Kumar E.G.T.V, Harinarayan C.V. 2002. Effect of ω-3 fatty acids on lipid peroxidation and antioxidant enzyme status in type 2 diabetic patients. Diabetes & Metabolism, Vol 28, N° 1 pp. 20-26.

Kris-Etherton PM, Zhao G, Binkoski AE, Coval SM, Etherton TD. 2001. The effects of nuts on coronary heart disease risk. Nutr Rev. 59:103-111.

Kulaksızoglu S and Karalezli A. 2016. Aqueous humour and serum levels of nitric oxide, malondialdehyde and total antioxidant status in patients with type 2 diabetes with proliferative diabetic retinopathy and nondiabetic senile cataracts. Can J Diabetes 40(2): 115-9.

Kumari N, Haider MR, Pathak A and Yar MS. 2019. Medicinal prospects of antioxidants. Eur J Med Chem; 178: 687-04.

Luvuno M, Khathi A and Mabandla MV 2021. Diet-induced prediabetes: effects of exercise treatment on risk factors for cardiovascular complications. Nutr Metab (Lond). 18(1): 45.

Madsen KS, Chi Y, Maria-Inti M, Richter B and Hemmingsen B. **2019**: Metformin for prevention or delay of type 2 diabetes mellitus and its associated complications in persons at increased risk for the development of type 2 diabetes mellitus. Cochrane Database Syst Rev ; 12.

Madsen KS, Kähler P, Kähler LKA, Madsbad S, Gnesin F, Maria-Inti M, Richter B, Hemmingsen B and Cochrane. 2019. Metabolic and endocrine disorders groupmetformin and second- or third-generation sulphonylurea combination therapy for adults with type 2 diabetes mellitus. Cochrane Database Syst Rev; 2019(4): CD012368.

Martınez-Gonzalez MA, Fernandez-Jarne E, Serrano-Martınez M, Wright M, Gomez-Gracia E. 2004. Development of a short dietary questionnaire for the quantitative estimation of adherence to a cardioprotective Mediterranean diet. Eur J Clin Nutr,;58: 1550-1552.

Meisinger C, Baumert J, Khuseyinova N, Loewel H, Koenig W. 2005. Plasma oxidized low-density lipoprotein, a strong predictor for acute coronary heart disease events on apparently healthy, middle-aged men from the general population. Circulation,;112:651-657.

Merzouk S, Hichami A, Sari A, Madani S, Merzouk H, Yahia and Berrouiguet A 2004. Impaired oxidant/antioxidant status and LDL-fatty acid composition are associated with increased susceptibility to peroxidation of LDL in diabetic patients. Gen Physiol Biophys; 23: 387- 99.

Mirmiran, P, et al. 2018. "Therapeutic lifestyle change diet enriched in legumes reduces oxidative stress in overweight type 2 diabetic patients: a crossover randomised clinical trial." European Journal of Clinical Nutrition, vol. 72, no. 1, Jan. 2018, p. 174.

Mitjavila MT, Fandos M, Salas-Salvado J, Covas M-I, Borrego S, estruch R, Lamuela-Raventos R, Corella D, Martinez-Gonzalez MA, Sanchez JM, Bullo M, Fito M, Tormos C, Cerda C, Casillas R, Moreno JJ, iradi A, Zaragoza C, Chaves J, Saez GT. 2012. The Mediterranean diet improves the systemic lipid and DNA oxidative damage in metabolic syndrome individuals. A randomized, controlled, trial. Clinical Nutrition 8 :1-7.

Mosbah.H, Sarazin.F, Zavras.S, Benoit.C, Ciangura.C, Bosquet.F, Hartemann.A, Andreelli.F 2018. Adhésion et effets métaboliques du régime méditerranéen dans le diabète de type 2. Hôpital Pitié Salpêtrière, service de diabétologie, Paris, France. Doi : 10.1016/j.ando.2018.06.880.

Mosenzon O, Prato SD, Schechter M, Leiter LA, Ceriello A, DeFronzo RA and Raz I. 2021. From glucose lowering agents to disease/diabetes modifying drugs: a "SIMPLE" approach for the treatment of type 2 diabetes. Cardiovasc Diabetol; 20(1): 92.

Natali A and Ferrannini E. 2006. Effects of metformin and thiazolidinediones on suppression of hepatic glucose production and stimulation of glucose uptake in type 2 diabetes: a systematic review. Diabetologia 2006; 49: 434- 41.

Ndrepepa G: Myeloperoxidase A. 2019. Bridge linking inflammation and oxidative stress with cardiovascular disease. Clin Chim Acta 2019; 493: 36-51.

Newsholme P, Cruzat VF, Keane KN, Carlessi R and de Bittencourt PIH. 2016. Molecular mechanisms of ROS production and oxidative stress in diabetes. Biochem J 2016; 473 (24): 4527-50.

Oguntibeju OO 2019: Type 2 diabetes mellitus, oxidative stress and inflammation: examining the links. Int J Physiol Pathophysiol Pharmacol 2019; 11(3): 45–6.

Oh DY, Talukdar S, Bae EJ, Imamura T, Morinaga H, Fan W, Li P, Lu WJ, Wtkins SM, Olefsky JM. 2010. GPR 120 is an omega-3 fatty acid receptor mediating potent anti-onflammatory and insulin-sensitizing effects. Cell 2010; 142:687-698.

Ola MS, Berkich DA and Xu Y. 2006. Analysis of glucose metabolism in diabetic rat retinas," The American Journal of Physiology: Endocrinology and Metabolism. 2006; 290(6): E1057–E1067.

Oleson BJ and John A. 2018. Corbett. Dual Role of Nitric Oxide in Regulating the Response of β Cells to DNA Damage. Antioxid Redox Signal 2018; 29(14): 1432-45.

Orhan H, Onderoglu L, Yücel A and Sahin G. 2003. Circulating biomarkers of oxidative stress in complicated pregnancies. Arch Gynecol Obstet 2003; 267: 189-195.

Owen RW, Mier W, Giacosa A, Hull WE, Spiegelhalder B, Bartsch H. 2000. Phenolic compounds and squalene in olive oils: the concentration and antioxidant potential of total phenols, simple phenols, secoroids, lignans and squalene. Food Chem Toxicol, 2000; 38:647-659.

Panagiotakos DB, Pitsavos C, Chrysohoou C, Skoumas J, Stefanadis C. 2004. Status and management of blood lipids in Greek adults and their relation to socio-demographic, lifestyle and dietary factors: the ATTICA Study. Blood lipids distribution in Greece. Atherosclerosis,
2004, 173:353–361.

Park CH and Jae WK 2012. Effect of Advanced Glycation End Products on Oxidative Stress and Senescence of Trabecular Meshwork Cells. Korean J Ophthalmo 2012; 26: 123-31.

Pisoschi AM, Pop A, Iordache F, Stanca L, Predoi G and Serban AI. 2021. Oxidative stress mitigation by antioxidants - An overview on their chemistry and influences on health status. Eur J Med Chem; 209: 112891.

Pitocco D, Zaccardi F, Di Stasio E, Romitelli F, Santini S A, Zuppi C and Ghirlanda G. 2010. Oxidative stress, nitric oxide, and diabetes. Rev Diabet Stud 2010; 7: 15-25.

Pomatto LCD and Davies KJA. 2018. Adaptive homeostasis and the free radical theory of ageing. Free RadicBiol Med 2018; 124: 420-30.

Poznyak A, Andrey V, Grechko, Poggio P, Myasoedova VA, Alfieri V and Orekhov AN 2020. The diabetes mellitus– atherosclerosis connection: the role of lipid and glucose metabolism and chronic inflammation. Int J Mol Sci 2020; 21(5): 1835.

Psaltopoulou T, Naska A, Orfanos P, Trichopoulos D, Mountokalakis T, Trichopoulou A 2004. Olive oil, the Mediterranean diet, and arterial blood pressure: the Greek European Prospective Investigation into Cancer and Nutrition (EPIC) study. Am J Clin Nutr, 2004, 80:1012–1018.

Rani V, Deep G, Singh RK, Palle K and Yadav UCS. 2016. Oxidative stress and metabolic disorders: Pathogenesis and Therapeutic Strategies 2016; 148: 183-93.

Richard C, Couture P, Desroches S, Lamarche B. 2013. Effect of the Mediterranean diet with and without weight loss on markers of inflammation in men with metabolic syndrome. Obesity 2013 ; 21 :51-57.

Roe JH and Kuether CA 1943. The determination of ascorbic acid in whole blood and urine through the 2, 4- dinitrophenylhydrazine derivatives of dehydroascorbic acid. J Biol Chem 1943; 147: 399-07.

Roma LP and Jean-Christophe J 2020 Nutrient Metabolism, Subcellular Redox State, and Oxidative Stress in Pancreatic Islets and β-Cells. J Mol Biol 2020; 432(5): 1461-93.

Rosca MG, Vazquez E J, Chen Q, Kerner J, Kern TS and Hoppel CL 2012: Oxidation of fatty acids is the source of increased mitochondrial reactive oxygen species production in kidney cortical tubules in early diabetes. Diabetes 2012; 61(8): 2074-83.

Santangelo C, Filesi C, Scazzocchio B et a. 2016. Consumption of extra-virgin olive oil rich in phenolic compounds improves metabolic control in patients with type 2 diabetes mellitus: a possible involvement of reduced levels of circulating visfatin. Journal of Endocrinological Investigation 2016 ; volume 39, pages1295–1301.

Santilli F, Cipollone F and Mezzetti A 2004. The role of nitric oxide in the development of diabetic angiopathy. Horm Metab Res 2004; 36: 319-35.

Sanz-Cameno P, Medina J, Garcia-Buey L, GarciaSanchez A, Borque MJ, Martin Vilchez S, Gamallo C, Jones EA and Moreno-Otero R. 2002. Enhanced intrahepatic inducible nitric oxide synthase expression and nitrotyrosine accumulation in primary biliary cirrhosis and autoimmune hepatitis. Journal of Hepatology 2002; 37- 723e729.

Schlienger JL, Monnier L 2020. Régimes méditerranéens et prévention du diabète : à l’heure des preuves. Médecine des Maladies Métaboliques. Volume 14, Issue 7, P.626-631.

Shah PK 2019. Inflammation, infection and atherosclerosis. Trends Cardiovasc Med 2019; 28: 468-72.

Shokrpour M, Foroozanfard F, Ebrahimi FA, Vahedpoor Z, Aghadavod E, Ghaderi A and Asemi Z. 2019. Comparison of myo-inositol and metformin on glycemic control, lipid profiles, and gene expression related to insulin and lipid metabolism in women with polycystic ovary syndrome: a randomized controlled clinical trial. Gynecol Endocrinol 2019; 35(5): 406-11.

St EO: 2021. Diabetes: Pharmacotherapy for Type 2 Diabetes. FP Essent 2021; 504: 22-27.

Stankova TR, Delcheva GT, Maneva AI, Vladeva SV 2019: Serum Levels of Carbamylated LDL, nitrotyrosine and soluble lectin-like oxidized low-density lipoprotein receptor-1 in poorly controlled type 2 diabetes mellitus. Folia Med (Plovdiv); 61(3): 419-25.

Summerhill VI, Grechko AV, Yet SF, Sobenin IA and Orekhov AN 2019 The atherogenic role of circulating modified lipids in atherosclerosis. Int J Mol Sci 2019; 20: 3561.

Taleb S 2016: Inflammation in atherosclerosis. Arch Cardiovasc Dis 2016; 109: 708-15.

Ting Y, Jia-Xing Z, Fa-Xuan W, Jian-Hua Z, Yu Z, Lan L, Xiu-Ying L, Yu-Hong Z, Yi Z. 2021. The Association between Sarcopenic Obesity and Hypertension, Diabetes, and Abnormal Lipid Metabolism in Chinese Adults. Diabetes Metab Syndr Obes 2021; 14: 1963-73.

Toobert DJ, Glasgow RE, Strycker LA, Barrera M, Radcliffe JL, Wander RC, Bagdade JD 2003 : Biologic and quality-of-life outcomes from the Mediterranean Lifestyle Program: a randomized clinical trial. Diabetes Care, 2003, 26:2288–2293.

Vaidya AR, Wolska N, Vara D, Mailer Rk, Schröder K And Pula G 2021 : diabetes and thrombosis: a central role for vascular oxidative stress. Antioxidants (Basel). 2021; 10(5): 706.

Van der PJ 20016 : Effect of lipid peroxidation on membrane permeability of cancer and normal cells subjected to oxidative stress. Chemical Science 2016; 7(1): 489-98.

Van SMF, de Graaf AA and Albert K 2018 : Groen.Actions of metformin and statins on lipid and glucose metabolism and possible benefit of combination therapy. Cardiovasc Diabetol 2018; 17: 94.

Vannereau et al. 2014 Consequences de la precarite sur le mode de vie et l’alimentation d’une population diabetique et pre-diabetique.. Medecine des maladies metaboliques 2014, 8(1) :88-94.

Velagic A, Qin C, Owen LL, Woodman, Horowitz JD and Rebecca H, Ritchie and Kemp-Harper BK 2020: Nitroxyl: a novel strategy to circumvent diabetes associated impairments in nitric oxide Signaling. Front Pharmacol 2020; 11: 727.

Vergès B 2001 : Insulinosensibilité et lipides. Diabetes & Metabolism 2001; 27-2-1262-36-36-01019-Art7.

Vergès B 2009: Lipid modification in type 2 diabetes: the role of LDL and HDL. Fundam Clin Pharmac 2009; 23(6): 681-5.

Wang Z, Wang Y, Liu H, Che Y and Xu Y 2022: Age-related variations of Protein carbonyls in human saliva and Rabehi et al., IJPSR, 2022; Vol. 13(1): 1000-09. E-ISSN: 0975-8232; P-ISSN: 2320-5148 International Journal of Pharmaceutical Sciences and Research 1007 plasma: Is saliva protein carbonyls an alternative biomarker of aging? Age Dordr 2015; 37: 81-97.

Xepapadaki E, Zvintzou E, Kalogeropoulou C, Filou S and Kypreos KE 2020 : The Antioxidant Function of HDL in Atherosclerosis. Angiology 2020; 71: 112–21.

Xiao-Bin H, Ting-Hui L, Zhi-Peng R and Liu Y 2016 : Combination of 2-deoxy d-glucose and metformin for synergistic inhibition of non-small cell lung cancer: A reactive oxygen species and P-p38 mediated mechanism. Biomed Pharmacother 2016; 84: 1575-58.

Yan L 2014 : Pathogenesis of chronic hyperglycemia: from reductive stress to oxidative stress. J Diabetes Res 2014; 14: 1-11.

Yaribeygi H, Sathyapalan T, Atkin SL and Sahebkar A 2020 : Molecular mechanisms linking oxidative stress and diabetes mellitus. Oxid Med Cell Longev; 2020: 8609213.

Zhou G, Myers R and Li Y 2001: Role of AMP-activated protein kinase in mechanism of metformine action. J Clin Invest; 108: 1167-74.

Zhuang T, Han H and Yang Z 2014: Iron, Oxidative Stress and Gestational Diabetes. Nutrients 2014; 6: 3968-80.

Zilov AV, Abdelaziz SI, AlShammary A, Zahrani AA, Amir A, Khalil SHA, Brand K, Elkafrawy N, Hassoun AAK, Jahed A, Jarrah N, Mrabeti S and Paruk I 2019: Mechanisms of action of metformin with special reference to cardiovascular protection. Diabetes Metab Res Rev 2019; 35(7): e3173.