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OXIDATIVE STRESS STATUS AND ADIPOKINES IN OBESE PATIENTS PRIOR TO METABOLIC SURGERY

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Summary

Background: Surgical treatments of obesity have demonstrated rapid improvements in insulin sensitivity and oxidative stress. The current study focuses on metabolic and oxidative stress differences between obese patients with or without type 2 diabetes mellitus (T2DM) prior and after metabolic surgery (Laparoscopic Sleeve Gastrectomy).

Objective: The aim of this preliminary study was to evaluate the oxidative stress status and adipokines in obese patients prior to metabolic surgery, in order to assess the differences between diabetic and non-diabetic patients.

Material and methods: 12 obese patients with T2DM and 17 non-diabetic obese subjects undergoing bariatric surgery were compared regarding anthropometric, clinical and oxidative stress markers. In all individuals we assessed BMI, waist and hip circumference, visceral fat index, serum levels of leptin, adiponectin, insulin and proinsulin. In order to evaluate the oxidative stress status of isolated peripheral blood mononuclear cells (PBMC) we measured the NADPH oxidase activity by luminol enhanced chemiluminescence ("respiratory burst" - RB) while their antioxidant activity was evaluated by measuring paraoxonase 2 (PON2) lactonase activity towards dihydrocoumarin (DHC). Total antioxidant capacity (TEAC), "Antioxidant gap" (GAP) and fructosamine levels were determined as well.

Results: All oxidative stress markers investigated were statistically significant modified for T2DM, except RB that do not differ in diabetic patients from those who are non-diabetic; they had decreased levels of TEAC ($p < 0.05$), adiponectin ($p < 0.05$) and PON2 ($p < 0.05$) and increased proinsulin ($p < 0.05$) and fructosamine ($p < 0.05$) levels. The obesity anthropometric markers were positively correlated with the values for leptin ($p < 0.05$) and negatively with the values for adiponectin ($p < 0.05$)

Résumé

Le statut du stress oxydatif et les adipokines chez les patients obèses avant la chirurgie métabolique

Introduction: Le traitement chirurgical de l'obésité a démontré une amélioration rapide de la sensibilité à l'insuline et le stress oxydatif. L'étude présent met l'accent sur les différences métaboliques et de stress oxydatif entre les patients obèses avec ou sans diabète sucré de type 2 (DS2) avant et après la chirurgie métabolique (gastrectomie longitudinale par ceolioscopie).

Objectif: L'objectif de cette étude préliminaire était d'évaluer l'état et de stress oxydatif les adipokines chez les patients obèses avant la chirurgie métabolique et afin d'établer les différences entre les patients diabétiques et non-diabétiques.

Matériel et méthodes: 12 patients obèses atteints de DS2 et 17 sujets obèses non diabétiques subissant une chirurgie bariatrique ont été comparés en ce qui concerne les données anthropométriques, les marqueurs de stress oxydatif et les données cliniques. Dans tous les individus, nous avons évalué l'index de masse corporelle, la circonférence de la taille et de la hanche, l'index de graisse viscérale, les niveaux de leptine, adiponectine, l'insuline et la pro-insuline sérique. Afin d'évaluer l'état de stress oxydatif des cellules périphériques isolées mononucléaires du sang périphérique (PBMC), nous avons mesuré l'activité de la NADPH oxydase par chimioluminescence amplifiée par luminol («burst respiratoire» - RB) alors que leur activité anti-oxydante a été évaluée par la mesure de la paraoxonase 2 (PON2) l'activité lactonase vers dihydrocoumarine (DHC). La capacité totale antioxydante (TEAC), «l'écart Antioxydant» (GAP) et les niveaux de fructosamine ont été déterminés egalement.

Résultats: Tous les marqueurs du stress oxydatif étudiés ont été statistiquement significativement modifiés pour le DS2, sauf RB qui ne diffère pas chez les patients diabétiques de ceux qui sont

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and this was consistent with the increased insulin and proinsulin levels ($p < 0.001$) in obese patients with T2D vs. non-diabetic subjects. PON2 lactonase activity was most correlated with diabetic parameters (glycaemia, fructosamine, proinsulin) and not with obesity ones (waist circumference, BMI, triglycerides).

Conclusion: The present study demonstrates a major disturbance of oxidant/ antioxidant balance in obese patients with T2DM. The increased oxidative stress in T2DM patients is nowadays considered to be the determinant factor that increases the chance for the onset of the metabolic syndrome. The influence of metabolic surgery over the oxidative stress in both cohorts is to be determined.

Key words: metabolic surgery, type 2 diabetes mellitus, paraoxonase 2, respiratory burst, oxidative stress.

non-diabétiques, Ils avaient une diminution des niveaux de TEAC ($p < 0,05$), l'adiponectine ($p < 0,05$), PON2 ($p < 0,05$), proinsuline ($p < 0,05$) et fructosamine ($p < 0,05$). Les marqueurs anthropométriques de l'obésité étaient positivement corrélés avec les valeurs de la leptine ($p < 0,05$) et négativement avec les valeurs de l'adiponectine ($p < 0,05$), ce qui était conforme aux niveaux accrus de l'insuline et de la proinsuline ($p < 0,001$) chez les patients obèses avec diabète de type 2 par rapport aux sujets non - diabetiques. L'activité de la lactonase PON2 a été la plus corrélée avec les paramètres diabétiques (glycémie, fructosamine, proinsuline) et non pas avec ceux de l'obésité (circonférence de taille, l'index de masse corporelle, triglycérides).

Conclusion: Cette étude démontre une perturbation majeure de l'équilibre oxydant / antioxydant chez les patients obèses atteints de DS2. Le stress oxydatif accru chez les patients diabétiques est aujourd'hui considéré comme le facteur déterminant qui augmente le risque de l'apparition d'un syndrome métabolique. L'influence de la chirurgie métabolique sur le stress oxydatif dans les deux cohortes est à déterminer.

Mots clés: chirurgie métabolique, diabète sucré de type 2, paraoxonase 2, stimulation du métabolisme oxydatif, stress oxydatif

INTRODUCTION

Defined as the excessive accumulation of adipose tissue caused by a chronic energy imbalance between energy expenditure and energy intake (1), obesity is one of the major priority global public health problem in the 21st century (2). Numerous longitudinal studies showed that obesity poses a significant risk factor on longevity, only one in seven obese individuals will reach the U.S. life expectancy of 76.9 years (3-5).

In obese patients, where lifestyle and medication have found not to be effective, bariatric surgery is the most effective procedure that causes a significant long-term weight loss, by reducing intake or absorption of calories. Moreover, bariatric surgery has been reported to reduce rates of cardiovascular disease (6) and to improve type 2 diabetes (7), through mechanisms beyond weight loss (8-10). Up to now, so-called hindgut hypothesis (11) and foregut hypothesis (9,12) have been advanced to explain the reversibility of diabetes.

In recent years the laparoscopic sleeve gastrectomy (LSG) raised significantly worldwide being proposed as an independent anti-obesity operation (13) offering a novel end point for diabetes: major improvement or even complete remission (3,14).

Oxidative stress, the imbalance between oxidants/ antioxidants in favor of pro-oxidant agents (15, 16) may play a critical role in the pathogenesis of obesity associated disease. Monocytes by means of their respiratory burst are an important source of free radicals that can promote atherogenic pathways in the vascular wall (17) and in diabetes monocytes display an enhanced NADPH oxidase activity, the enzyme responsive for respiratory burst (18). Unlike PON1, the activity of intracellular antioxidant enzyme paraoxonase 2 (PON2) increases in response to oxidative stress, probably through a compensatory mechanism against the oxidative stress (19).

We are conducting a study (C.R.E.D.O.R. - Collaborative Romanian Efforts for Diabetes and Obesity Retrench) that follows the metabolic and oxidative stress differences between obese patients with or without T2DM prior and after metabolic surgery (laparoscopic sleeve gastrectomy). The aim of this preliminary study was to evaluate the oxidative stress status and adipokines in obese patients prior to metabolic surgery, in order to assess the differences between diabetic and non-diabetic patients.

MATERIAL AND METHODS

Study design

A total of 29 obese patients (18 females, 11 males; BMI > 35 Kg/m2) enrolled into the bariatric surgery program of Ponderas Hospital were selected to participate in this study: 12 of them were diagnosed with type 2 diabetes and 17 were non-diabetic obese subjects.

The inclusion criteria were obese patients with T2DM less than 10 years from onset. 17 subjects proposed for bariatric surgery were recruited as control group, none of them having any suspicion of diabetes or glucose intolerance (normal glycaemia as well as HbA1c $< 5.9\%$). T2DM was diagnosed according to the WHO (World Health Organization) criteria, as follows: (1) serum fasting glucose $\geq$ 7.0 mmol/L (126 mg/dL), (2) 2 hour serum glucose obtained after an oral glucose tolerance test (OGTT) $\geq$ 11.1 mmol/L (200 mg/dL), (3) an HbA1c $\geq$ 6.5%, (4) a random plasma glucose $\geq$ 200 mg/dL (11.1 mmol/L) (20, 21).

The exclusion criteria for all subjects were: type 1 diabetes, previous metabolic surgery, gastro-intestinal or colic resection, psychiatric disorders or prior malignancies, excessive alcohol consumption (> 20g/day).

The study was approved by the Ethics Committee of Ponderas Hospital and all participants signed the informed consent before undergoing bariatric surgery.

Blood analysis

The day before surgery, after an overnight fast, blood samples were collected into vacuum tubes with no or EDTA-containing and immediately used for isolation of peripheral blood mononuclear cells (PBMC) while the remained sample (no EDTA-containing) was stored at -80°C until required.

Anthropometric measurements

Anthropometric measurements included weight, height and waist circumference (WC), were done by using a bio-electrical impedance analyzer (Tanita Body Composition Analyzer MC-980 -Tanita Corporation of America, Inc. IL, USA).

Routine biochemical analyses

Routine biochemical analyses including the concentration of fasting glucose, HbA1c, total cholesterol, HDL-cholesterol ("high density lipoprotein"; HDL-c), total triglycerides (TG), urea, creatinine, uric acid were measured using current biochemical methods on a Hospitex Diagnostics Eos Bravo Forte Analyzer. LDL-cholesterol ("low density lipoprotein"; LDL-c) was calculated according to the Friedewald equation (22), BMI as Kg/m^2 and HOMA-IR ("Homeostasis Model for Insulin Resistance") as [glycaemia (mmol /L) x insulinemia (μU/mL)]: 22.5 (23). Serum concentrations of insulin, proinsulin, leptin and adiponectin were determined by ELISA method on Multiskan Ex-Thermo Electro Corporation using commercially available kits EIA-2935 (CV 2.2%), EIA-1560 (CV 4.86%), EIA-2395 (CV 6.43%) and respectively EIA-4177 (CV 5.66%); DRG Instruments GmbH, Germany) while for fructosamine the CAIMAN kit (CV 5%) was used following for all the manufacturer's guidelines.

Trolox Equivalent Antioxidant Capacity (Plasma total antioxidant capacity; TEAC) and plasma residual Anti-oxidant Activity ("antioxidant Gap; GAP)

TEAC was made according to the Miller et al. method (24) with modifications (25) while GAP was calculated by subtracting the antioxidant capacity of albumin and uric acid from the total TEAC value, according to the formula: GAP=TEAC-[(albumin (mmol/L)X0.69 + uric acid (mmol/L)X1.0)], where 0.69 and 1.0 are the TEAC values for humans serum albumin and serum uric acid, respectively (24, 26). Briefly, the TEAC assay measures the relative abilities of antioxidants to scavenge the 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation (ABTS*+) generated from the interaction between ABTS and potassium per sulphate. The assay was calibrated against a calibration curve with the water soluble vitamin E analogue Trolox (6-hydroxy-2, 5, 7, 7-tetramethylchroman-2-carboxylic acid) as standard. 10μL plasma samples were added to the solution containing ABTS*+ and the absorbance was read after 1 minute at 734nm against 5mM phosphate buffer (pH7.4). The percentage inhibition of the absorbance was calculated and the results were expressed as mmol/L or mMTrolox.

Oxidant and antioxidant capacity of isolated peripheral blood mononuclear cells (PBMC)

PBMC were isolated by density centrifugation on Ficoll-Paque™ Plus (1.0077g/mL) at 630g for 30 minutes. After cell viability by Trypan Blue exclusion was done (always ≥ 90%), the ability to produce a RB was monitored by luminol-enhanced chemiluminescence method (27). In short, to PBMC (0.3 x 106 cells) washed twice and re-suspended in 1mL PBS, dark-adapted luminol was added. After monitoring spontaneous chemiluminescence for 15 min, the RB was initiated by adding of 100µL phorbol 12-myristate 13-acetate (PMA) (final concentration 5.4 μmol/L) and the maximum chemiluminescence peak was recorded using LuminoskanAscent® 392 device (LabsystemsEx-ThermoElectro Corporation). Chemiluminescence production was expressed as the Relative Chemiluminescence Units over time (RLUX60min).

After protein concentration was determined using the method of Bradford (28) paraoxonase 2 lactonase activity was measured on triplicate samples towards dihydrocoumarin at 270nmusing a continuously recording CECIL-CE 1010 UV/VIS spectrophotometer. PBMC cells (30x103) after collection were washed (2X) with Dulbecco's Phosphate Buffered Saline(PBS) and enzyme activities were measured using 1000μg protein/mL by adding 200μL cells to 1800μL Tris buffer (25 mmol/L Tris/HCl, 1 mmol/L CaCl2, pH 7.6) containing 1mmol/L DHC. One unit of lactonase activity is equal to 1 μmol of DHC hydrolyzed/mL/min using the extinction coefficient of 1295M-1 cm-1 (29).

All reagents, Dulbecco's Phosphate Buffered Saline (PBS), Ficoll-Paque™ Plus, TrypanBlue, phorbol 12-myristate 13-acetate (PMA), luminol (5-amino-2,3-dihydro-1,4-phthalazinedione; LM), 6-hydroxy-2,5,7,7-tetramethylchroman-2-carboxylic acid (Trolox),

2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), potassium persulphate and dihidrocoumarine (DHC) were purchased from Sigma Chemical Co., St. Louis, MO, USA.

Statistical Analysis

Results were expressed as mean ± standard deviation (SD)/standard error of the mean (SEM). Data were collected and introduced in an Apache OpenOffice database, version 4.1.0 (Copyright © 2014 The Apache Software Foundation). Statistical analysis was performed using R program, version 3.1.2 (2014-10-31) -- "Pumpkin Helmet" (c) R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria URL http://www.R-project.org/.).

Statistical algorithm was as follows: (1) if distribution of variables could be approximated by a normal undergo a Student's t Two-sided test with Satterthwaite approximation for unequal variants, (2) if distribution of variables

could not be approximated by a normal (Skewnes value distributions were significantly different from 0, were detected outlier in the Box plot) Wilcoxon rank sum test was used.

Results and Discussion

The patients' biochemical, oxidative stress and ELISA results are shown in Table 1. There were no significant differences between the studied groups regarding age, weight, waist circumference and body mass index. Comparing the groups regarding the lipid profile only the value for serum triglycerides was significantly increased in diabetic patients. No significant differences were recorded between diabetic and non-diabetic patients regarding urea, creatinine, uric acid, AST, ALT and GGT levels.

Diabetic patients had increased levels of proinsulin ($p < 0.05$) and fructosamine (542 ± 87 μmol/L vs. 372 ± 80 μmol/L, $p < 0.05$) and lower levels of PON2 (0.02 ± 0.001 vs. 0.14 ± 0.019

U/mg proteins, $p < 0.05$) and adiponectine ($p < 0.05$) when compared to the non-diabetic patients.

Table 1 - The clinical, oxidative stress and ELISA results of non-diabetic versus diabetic patients

Variable	Non-diabetic Subjects	Diabetic Patients	p Value [CI95%]
Age (years) mean (±SD)	40.94 (±11.57)	49.25 (±9.23)	0.0705[1] [-17.39 to 0.77]
WC (cm) median(IQR)	128.00 (22.50)	118.00 (8.00)	0.3694[2] [-]
BMI (Kg/m^2) median(IQR)	41.22 (15.05)	39.79 (15.44)	0.6460[2] [-]
Glycemia (mg/dL) mean(±SD)	93.07 (±16.56)	169.28 (±31.35)	0.0004[1] [-105.60 to -46.85]
HbA1c (%) median(IQR)	5.73 (0.21)	6.97 (2.48)	0.0001[2] [-]
Serum total Cholesterol (mg/dL) mean(±SD)	208.91 (±28.04)	210.83 (±65.38)	0.9475[1] [-70.33 to 66.50]
Serum HDL Cholesterol (mg/dL) median(IQR)	45.65 (19.80)	45.05 (17.80)	0.6734[2] [-]
Serum LDL Cholesterol (mg/dL) mean (±SD)	126.45 (±21.57)	120.51 (±39.32)	0.7409[1] [-35.35 to 47.23]
Triglycerides (mg/dL) mean (±SD)	148.10 (±58.25)	229.00 (±58.03)	0.0143[1] [-142.70 to -19.06]
Urea (mg/dL) mean (±SD)	33.66 (±8.65)	40.33 (±10.09)	0.2006[1] [-17.60 to 4.27]
Creatinine (mg/dL) mean (±SD)	0.98 (±0.17)	0.95 (±0.15)	0.7018[1] [-0.12 to 0.18]
Uric Acid (mg/dL) mean (±SD)	6.38 (±1.25)	6.55 (±1.04)	0.7707[1] [-1.38 to 1.05]
Albumin (mg/dL) mean (±SD)	4.62 (±0.24)	4.60 (±0.22)	0.7929[1] [-0.20 to 0.25]
ALT (U/mL) median (IQR)	25.00 (12.00)	24.00 (8.00)	0.7936[2] [-]
GGT (U/mL) median (IQR)	31.00 (14.00)	32.00 (13.00)	0.9702[2] [-]
Fibrinogen (mg/dL) mean (±SD)	372.80 (±95.07)	542.87 (±176.36)	0.0451[1] [-335.30 to -4.80]
HOMA-IR median (IQR)	2.19 (1.39)	4.83 (4.93)	0.0093[2] [-]
Insulin (μIU/mL) median (IQR)	9.45 (5.15)	10.16 (14.61)	0.3746[2] [-]
Proinsulin (pmol/L) median (IQR)	3.04 (3.09)	5.83 (13.21)	0.0077[2] [-]
C peptide (ng/mL) median (IQR)	3.17 (3.33)	4.73 (2.67)	0.2838[2] [-]
Leptin (ng/mL) mean (±SD)	33.88 (±27.87)	36.02 (±21.21)	0.8364[1] [-23.65 to 19.36]
Adiponectin (μg/mL) median (IQR)	8.68 (3.68)	4.14 (3.29)	0.0297[2] [-]
RB (RLU) median (IQR)	0.02 (0.02)	0.02 (0.04)	0.6241[2] [-]
PON2 (U/mg protein) mean (±SD)	0.14 (±0.06)	0.02 (±0.03)	< 0.0001[1] [0.07 to 0.16]

WC - Waist Circumference AST - aspartat aminotransferase; ALT – alaninamino-transferase; GGT – gamma glutamyltransferase;
RB – Respiratory Burst of the PBMC, PON2 – Paraoxonase 2 lactonase activity of the PBMC
[1]Two –sample Student's t test two –sided with Satterthwaite approx. for unequal variances; [2]Wilcoxon rank sum test

PON2 levels were more correlated with diabetic parameters as fructosamine (p 0.0013), HbA1c (p 0.0.004) and glycemia (p 0.0165) than with obesity markers as waist circumference (p 0.1900), BMI (p 0.9438) and triglycerides (p 0.2693).

The obesity anthropometric markers were positively correlated with the values for leptin ($p < 0.05$) and negatively with the values for adiponectin ($p < 0.05$) and this was consistent with the increased insulin and proinsulin levels ($p < 0.001$) in obese patients with T2DM vs. non-diabetic subjects.

As shown in Fig. 1, the plasma total antioxidant capacity (TEAC) decreased in the T2DM group compared with non-diabetic patients (1.45±0.03 mM vs 1.24±0.06 mM, $p < 0.05$) while plasma residual Antioxidant Activity (GAP) did not differ between the two studied groups (data not shown).

Exploring whether there are correlations between different parameters for PON2 levels and patient characteristics (obesity and type 2 diabetes) we calculated Spearman and Pearson correlation indices (based on distributions of variables) (Table 2).

We constructed a linear regression model using the response variable (dependent variable) PON2 and as predictors (independent variables) parameters that have a significant correlation index and had a distribution that could be approximated by a normal one (to meet the requirements for linear regression model). The model was as follows PON2 = FRZ (fructosamine) + GLY (glycaemia) + FRZ:GLY and the results we obtained are shown in Table 3.

In the model we presented there is a period of interaction between glycaemia and fructosamine because the correlation between them (Pearson Index 0.3089). The model does not contain HbA1c and proinsulin because although indices were significantly correlated (-0.7755 -0.5415 respectively),

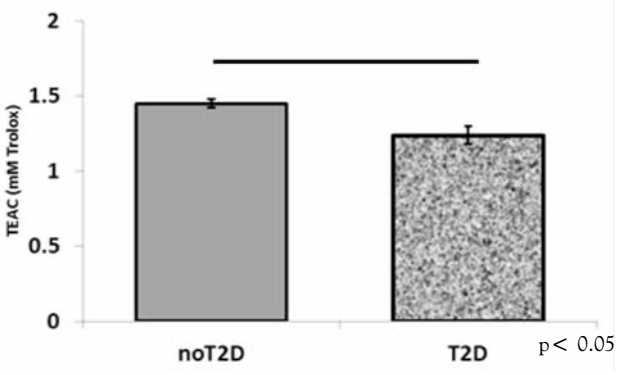

Figure 1 - Plasma total antioxidant capacity (TEAC) non-diabetic versus diabetic patients

distributions of these parameters could not be considered normal distributions. All model coefficients are different from 0 and R^2 is a significant value (0.6920).

PON2 levels were more correlated with diabetic parameters as fructosamine ($p = 0.0013$), HbA1c ($p = 0.0.004$) and glycemia ($p = 0.0165$) than with obesity markers as waist circumference ($p = 0.1900$), BMI ($p = 0.9438$) and triglycerides ($p = 0.2693$).

To investigate whether oxidative stress balance is disturbed in obese subjects undergoing LGS, we measured on the one hand respiratory burst intensity in PBMC and on the other hand paraoxonase 2 (PON2) lactonase antioxidant activities. We found a lower PON2 in all obese subjects independently of the presence of diabetes while the activity of one of the most important reactive oxygen species producers, the enzyme NADPH oxidase, which generates superoxide anions during the phagocyte RB process (30) was not significantly modified when compared the two groups. It is well known that insulin and cytokines act on the enzyme NADPH oxidase stimulating H2O2 production and high

Corelations	Pearson / Spearman coefficient	p value [CI95%] for Pearson coefficient
PON2 vs Glycaemia	-0.5716[1]	0.0165 [-0.8254 to -0.1254]
PON2 vs Fructosamine	-0.6811[1]	0.0013 [-0.8670 to -0.3285]
PON2 vs C Peptide	-0.2591 -0.1168[2]	0.2566 0.6127
PON2 vs Proinsulin	-0.5415[2]	0.0124
PON2 vs Insulin	0.0753[2]	0.7454
PON2 vs BMI	0.0168[2]	0.9438
PON2 vs Waist Circumference	0.3722[2]	0.1900
PON2 vs HbA1c	-0.7755[2]	0.0004
PON2 vs Leptin	0.0886[1]	0.7024 [-0.3566 to 0.5011]
PON2 vs Adiponectin	0.1532[2]	0.5056
PON2 vs HOMA-IR	-0.3883[2]	0.0828
PON2 vs Triglycerides	-0.3048[1]	0.2693 [-0.7067 to 0.2458]
PON2 vs LDL	0.0612[1]	0.8283 [-0.4656 to 0.5560]
PON2 vs VLDL	-0.2464[2]	0.3748
PON2 vs HDL	0.1535[2]	0.5844

[1] Pearson's product-moment correlation [2] Spearman's rank correlation rho

Table 2 - Spearman and Pearson correlation indices (based on distributions of variables) between different parameters and PON2 levels

Table 3 - Linear regression model using the response variable (dependent variable) PON2 and as predictors (independent variables) glycemia and fructosamine parameters

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	0.8632	0.2108	4.09	0.0015
FRZ	-0.0016	0.0005	-3.17	0.0081
GLY	-0.0049	0.0016	-3.02	0.0107
FRZ:GLY	0.000009	0.000003	2.59	0.0235
R^2	Adj - R^2	Root MSE	PON2 Mean	
0.7535	0.6920	0.0504	0.0998	

NADPH oxidase activity, in turn, increased the risk of insulin resistance (Eriksson JW., 2007). On the other hand, PON2 enzyme presented in macrophages is able to lower the intracellular oxidative stress of this cell, PON2 mRNA being up-regulated in response to oxidative stress probably as a compensatory mechanism against oxidative stress (19).

Importantly, in the present study patients with T2DM were shown to have lower antioxidant PON2 activity than non-diabetic subjects. It is argued that all the postoperative improvements in metabolic status of these patients will concur to the remission/improvement of T2DM.

Lastra G. (31) demonstrated that dysfunctional adipose tissue in obese patients is responsible for the low-grade inflammation that spreads to several tissues as well as systemically, being able to impact even the cardiovascular system. The inappropriately activated renin-angiotensin-aldosterone system (RAAS) in adipose tissue will increase production of reactive oxygen species (ROS), which will cause resistance to the metabolic actions of insulin in several tissues.

A study by Hulsmans M. (32) determined the effect of weight loss after metabolic surgery, showing that IRAK3 (Interleukin-1 receptor-associated kinase-3), a key inhibitor of chronic inflammation, is down-regulated in monocytes of obese patients. Marfella R. (33) showed that oxidative stress reduction after biliopancreatic diversion seems to be related to the regulation of glucose fluctuations resulting from intestinal bypass.

Further studies, including C.R.E.D.O.R. (Collaborative Romanian Efforts for Diabetes and Obesity Retrench), which is due to be finished in 2016, may demonstrate that this decreased antioxidant defense in patients with obesity and T2DM could be significantly improved after LSG.

Conclusions

The present study demonstrates a major disturbance of oxidant/ antioxidant balance in obese patients with T2DM, which is considered to be the major risk factor for developing metabolic syndrome and increased morbidity and mortality. As any other weight loss method, conservative or surgical, the LSG is expected to act on these alterations, improving the oxidative/antioxidant status, thus remitting /improving theT2DM.

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Autor's contribution

Dr. B. Smeu and Dr. D. Lixandru had equal contribution to this paper.

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