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Susceptibility to oxidative stress, insulin resistance, and insulin secretory response in the development of diabetes from obesity

Osetljivost na oksidativni stres, insulinsku rezistenciju i insulinsku sekretornu reakciju u razvoju dijabetesa iz gojaznosti

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Abstract

Background/Aim. Oxidative stress plays a critical role in the pathogenesis of various diseases. Recent reports indicate that obesity may induce systemic oxidative stress. The aim of the study was to potentiate oxidative stress as a factor which may aggravate peripheral insulin sensitivity and insulin-secretory response in obesity in this way to potentiate development of diabetes. The aim of the study was also to establish whether insulin-secretory response after glucagon-stimulated insulin secretion is susceptible to prooxidant/antioxidant homeostasis status, as well as to determine the extent of these changes. **Methods.** A mathematical model of glucose/insulin interactions and C-peptide was used to indicate the degree of insulin resistance and to assess their possible relationship with altered antioxidant/prooxidant homeostasis. The study included 24 obese healthy and 16 obese newly diagnozed non-insulin dependent diabetic patients (NIDDM) as well as 20 control healthy subjects, matched in age. **Results.** Total plasma antioxidative capacity, erythrocyte and plasma reduced glutathione level were significantly decreased in obese diabetic patients, but also in obese healthy subjects, compared to the values in controls. The plasma lipid peroxidation products and protein carbonyl groups were significantly higher in obese diabetics, more than in obese healthy subjects, compared to the control healthy subjects. The increase of erythrocyte lipid peroxidation at basal state was shown to be more pronounced in obese daibetics, but the apparent difference was obtained in both the obese healthy subjects and obese diabetics, compared to the control values, after exposing of erythrocytes to oxidative stress induced by H_2O_2. Positive correlation was found between the malondialdehyde (MDA) level and index of insulin sensitivity (FIRI). **Conclusion.** Increased oxidative stress together with the decreased antioxidative defence seems to contribute to decreased insulin sensitivity and impaired insulin secretory response in obese diabetics, and may be hypothesized to favour the development of diabetes during obesity.

Key words:
oxidative stress; diabetes mellitus, type 2; obesity; disease progression.

Apstrakt

Uvod/Cilj. Oksidativni stres igra važnu ulogu u patogenezi raznih bolesti. Najnovija saopštenja ukazuju da gojaznost može izazvati sistemski oksidativni stres. Cilj ovog rada bio je da se ukaže na važnost oksidativnog stresa kao faktora koji može negativno da utiče na perifernu insulinsku osetljivost i insulinsku sekretornu reakciju kod gojaznosti i na taj način izazove razvoj dijabetesa. Cilj rada takođe je bio i da se utvrdi da li je insulinska sekretorna reakcija posle glukagon-stimulisane insulinske sekrecije osetljiva na prooksidativni/antoksidativni status homeostaze i da se odredi obim promena. **Metode.** Matematički model interakcija glukoza/insulin i C-peptida uzet je kao pokazatelj stepena insulinske rezistencije i utvrđivanja njihove moguće veze sa izmenjenom antioksidativnom/prooksidativnom homeostazom. Studija je obuhvatala 24 gojazne zdrave osobe i 16 gojaznih sa novopostavljenom dijagnozom insulin-nezavisnog dijabetesa (NIDDM), kao i 20 zdravih osoba kontrolne grupe usklađenih po starosti. **Rezultati.** Značajno su bili sniženi ukupni plazma antioksidativni kapacitet i redukovani nivoi glutationa eritrocita i plazme kod gojaznih bolesnika sa dijabetesom, ali i kod zdravih gojaznih osoba u poređenju sa kontrolnom grupom. Proizvodi lipidne peroksidacije i karbonilne grupe proteina u plazmi bili su značajno viši kod gojaznih sa dijabetesom nego kod zdravih gojaznih osoba u poređenju sa kontrolnom grupom zdravih osoba. Porast lipidne peroksidacije eritrocita u bazalnom stanju bio je izraženiji kod gojaznih sa dijabetesom, ali je uočljiva razlika postojala i u grupi gojaznih zdravih osoba u poređenju sa vrednostima u kontrolnoj grupi posle izlaganja eritrocita oksidativnom stresu indukovanom pomoću H_2O_2. Nađena je pozitivna korelacija između nivoa malondialdehida (MDA) i indeksa insulinske osetljivosti (FIRI). **Zaključak.** Povišeni oksidativni stres zajedno sa sniženom antioksidativnom zaštitom izgleda da doprinosi sniženju insulinske osetljivosti i insulinske sekretorne reakcije kod gojaznih bolesnika sa dijabetesom, te se može pretpostaviti da ubrzava razvoj dijabetesa kod gojaznih osoba.

Ključne reči:
stres, oksidativni; dijabetes melitus, insulin-nezavisni; gojaznost; bolest, progresija.

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Introduction

Oxidative stress plays a critical role in the pathogenesis of various diseases [1]. Recent reports indicate that obesity may induce systemic oxidative stress [2]. Increased free radical production in accumulated fat, because of increased nicotinanide adenine dinucleotide phosphate (NADPH) oxidase and decreased antioxidant enzymes activity, causes dysregulated production of adipocytokines locally, such as plasminogen activator inhibitor–1 (PAI-1), tumor necrosis factor α (TNF-α), resistin, leptin and adiponectin [3–8]. Increased oxidative stress in blood can affect other organs such as endothelium, liver and skeletal muscle, and may lead to decreased insulin sensitivity and development of insulin resistance in obese subjects [8–10].

In diabetes, oxidative stress impairs glucose uptake in muscle and fat and decreases insulin secretion from pancreatic β cells [11–13]. Increased oxidative stress also underlies the pathophysiology of late diabetic complications directly affecting vascular wall [14].

The life time risk to develop impaired glucose tolerance and diabetes is increased among obese subjects compared to normal-weight matched subjects [15, 16]. Complex interactions between obesity and insulin resistance largely account for the pathogenesis of non-insulin dependent diabetes. There are many factors that can aggravate insulin resistance of obesity. Elevated insulin level itself is atherogenic by stimulating sympathetic nerve activity. Increased insulin secretion is associated with the progressive loss of β-cell function as well. Insulin resistance results in reduced peripheral disposal of glucose in muscle and increased hepatic glucose output in the fasting state [17, 18]. Decreased concentration of reduced thiols together with increased lipid peroxidation products, may play a role in the development of insulin resistance, because an optimal antioxidant/prooxidant state is required for the stabilization of nitric oxide available for insulin action [19, 20].

The study was performed with the aim to estimate the relation between susceptibility to oxidative stress and the antioxidative defense system as possible risk factors of importance in the development of diabetes during obesity; to establish whether insulin-secretory response was susceptible to prooxidant/antioxidant status of the homeostasis, the extent of their changes in the course of stimulated insulin secretion was performed. A mathematical model of glucose/insulin interactions was used to indicate the degree of insulin resistance and to asses the possible relationship with the altered antioxidant/prooxidant homeostasis.

Methods

The study included 24 obese healthy and 16 obese newly diagnozed non-insulin dependent diabetic patients (NIDDM), as well as 20 control healthy subjects, blood donor volunteers, matched in age. The degree of obesity was defined in terms of body mass index–BMI (kg/m^2). Patients and control subjects taking antioxidative vitamins or other drugs with antioxidative properties were excluded from the study.

Heparinized venous blood was obtained by peripheral venopuncture. The first blood sample was always collected at 8.00 am after 12 h of fasting, the second was collected after the glucagon test. All samples were immediately centrifuged at 2 000g for 15 min, plasma was carefully removed. Sedimented erythrocytes were washed three times in physiological saline. Received plasma and erythrocytes were immediately processed for the analyses described below.

All chemicals were of analytical grade purity, including 5.5 dithiobis nitrobensoic acid – DTNB, thiobarbituric acid – TBA and xanthine (ICN Biochemicals, Costa Mesa, CA USA and Serva).

Residual β-cell function was assessed by using the intravenous glucagon stimulation test. Obese subjects and obese diabetics received 1mg intravenously (i.v.) glucagon and blood samples were collected at basal state (0 min) and 6 min after. The values for the residual β-cell function was assessed from the insulin and glucose concentrations by the formula [21]:

β-cell function (%) = 20 × insulin/glucose-3.5 (before and after the glucagon stimulation).

Insulin resistance was expressed as an empirical fasting insulin resistance index (FIRI) consisting of the product of plasma insulin and glucose that was obtained by the formula [22]:

FIRI = fasting glucose × fasting insulin/25 (normally around 1 unit).

The reduced glutathione level (GSH) in plasma was measured by spectrophotometric assay using DTNB [23]. The level of plasma GSH was expressed as μmol/l.

The packed erythrocytes (0.2 ml) were treated with the same volume of ice-cold H_2O and 1.3 ml of 5% TCA-1.0 mM EDTA immediately after the drawing. The supernatant was analyzed for sulphydryl group content at 412 nm with DTNB in 0.1 M phosphate buffer [23]. The level of erythrocyte reduced glutathione (RBC GSH) was expressed as μmol/ml RBC.

Plasma antioxidative capacity was tested by the modification of procedure of Asakawa and Matsushita [24] using an *in vitro* model of Fe^{2+}-induced peroxidation of liposome suspension (from egg yolks). For this purpose 1.5 ml of liposome suspension at the concentration of 0.5 mg of total egg yolk phospholipids/ml, 0.2 ml of 0.5 M phosphate buffer *pH* 7.4 and 0.3 ml of tested plasma sample were incubated at 37 ^oC for 30 min by adding 0.2 ml of 1 mM/l $FeCl_2$. Then 1 ml of 30% TCA was added to the reaction mixture followed by centrifugation for 15 min at 10 000 g. One milliliter of 0.67% TBA was added to 1ml of the received supernatant and the mixture was boiled at 97 ^oC for 20 min. Optical absorbance was measured at 532 nm. In this way, under prooxidant conditions (in the presence of traces of iron) incubation was carried out and the capacity of plasma to scavenge free oxygen radical production was determined as malondialdehyde (MDA) level. The antioxidative capacity was expressed as the percent of inhibition of MDA formation com-

pared with the control samples which contained phosphate buffer instead of plasma.

Plasma lipid peroxidation was determined by thiobarbituric acid (TBA) reactivity. A colored complex has a maximum absorbance at 532 nm. The level of MDA was expressed as μmol/l. The activity of xanthine oxidase was determined by the increase of uric acid production when xanthine was used as substrate [25].

The level of lipid peroxidation products in erythrocytes was estimated by measuring TBA reactivity [26]. For this purpose 0.4 ml of packed cells were suspended in 1.6 ml of phosphate buffer saline *pH* 7.4 (as a 20% suspension of red cells). To this suspension 0.5 ml of 30% TCA was added and 2 ml of the obtained supernatant was transferred into another tube, and 0.15 ml of 0.1 M/l EDTA and 0.5 ml of 1% TBA (dissolved in 0.05 M NaOH) was added. Tubes were mixed and kept in a boiling water for 15 minutes. After thad the tubes were cooled to room temperature, absorbance was read at 532 nm and 600 nm. Absorbance at 600 nm was substracted from absorbance at 532 nm. Malondialdehyde values in nmol/ml of erythrocytes (RBC) were determined using the extinction coefficient of MDA/TBA complex at 532 nm (1.56×10^5 per cm per molar solution). The results were expressed as nmol MDA/ml RBC.

For estimation of erythrocyte susceptibility to H_2O_2 induced oxidative stress, erythrocytes were washed with an isotonic saline solution containing 2.0 mM sodium azide to inhibit catalase activity [26]. Washed erythrocytes were resuspended in saline-azide potassium phosphate buffer *pH* 7.4. One mililiter of 20% cell suspension was then preincubated for 10 min at 37 °C. Peroxidative challenge was induced by addition of an equal volume (1.0 ml) of H_2O_2 in isotonic saline-azide solution (final H_2O_2 concentration 3.0 mM). The reaction was terminated by the addition of 0.5 ml of 30% TCA and mixture was centrifuged. Malondialdehyde was detected in the obtained supernatant as a basic TBA reacting agent and it was used as an indicator of lipid peroxidation. The obtained results were expressed as nmolMDA/ml RBC.

Protein carbonyls were measured in plasma proteins and the remaining protein pellet according to the method explained in our previous report [27].

Heparinised blood samples were collected for the assay procedures. After preparing the hemolysate, where the labile fraction is eliminated, hemoglobins are retained by cationic exchange resin and chromatographic (ion exchange)-spectrophotometric method was used for the measurement of hemoglobin A_1c (BioSystems). Plasma fructosamine was measured by the method of Bake et al [28].

Plasma glucose was monitored by an ILAB 300 analyzer.

Plasma C-peptide and insulin were assayed by using the radioimmunoassay kits (Inep- Vinca-Serbia).

All presented values were given as mean ± SD for statistical evaluation of the results between two groups. For multiple comparisons of means the ANOVA analysis of variance was used. The coefficient of the correlation (r) between the investigated parameters was obtained by the linear regression analysis and by the regression equation.

Results

The clinical characteristics of 24 obese healthy subjects and 16 obese – newly diagnosed diabetics, at the time of the study, are shown in Table 1. The mean age of obese subjects was 48.52±6.4 yr (mean ±SD; range 36–61 yr) and of obese diabetics 50.3±6.2 yr (range 42–61 yr). There was a predominance of female (n = 17) over male obese subjects (n = 7) and of obese diabetics 9 were male and 7 female. Plasma HbA1c and fructosamine level were significantly higher in obese diabetics compared with the level in obese healthy subjects. Significant difference was found between functional activity of β-cells (expressed as percents of C-peptide secretion during normal function). The obtained results point to significantly reduced residual β-cell function in obese diabetics at basal state and after glucagon load (Figures 1 and 2).

Table 1

Main clinical characteristics of obese healthy subjects and obese recent-onset non-insulin dependent diabetes mellitus (NIDDM) patients

Variables	Obese healthy subjects mean ±SD	Obese NIDDM patients mean ±SD	Control subjects mean ±SD
Number of patients	24	16	20
Sex (male/female)	7/17	9/7	12/8
Age (yr)	48.52±6.4	52.3±6.7	44.33±5.3
Body mass index (kg/m^2)	34.33±4.2*	33.1±5.1*	25.32±4.34
Duration of diabetes (months)	–	7.8±5.4	–
Plasma fructosamine (U/g proteins)	0.44±0.1	0.72±0.2*	0.39±0.07
Plasma hemoglobin A1c (g/l)	4.3±0.7	11.9±4.0	4.0±1.1
Fasting plasma glucose (mmol/l)	4.8±0.8	12.3±3.6*	4.2±0.5

* $p < 0.001$

Significantly lower total plasma antioxidative capacity was found in both groups of the obese diabetics and the obese healthy subjects compared with the control values (Figure 3). The values of total plasma antioxidative capacity, as well as plasma and erythrocyte GSH (glutathione) concentration progessively decreased from obese healthy sub-

jects to obese diabetics (Figures 3 and 4). The level of plasma proten oxidative modification products-protein carbonyls was significantly increased in both groups compared to controls (Figure 4). Plasma MDA level of obese diabetics had the highest value, but in obese healthy subjects it was also increased compared with the control values. Statistically significant increase in the concentrations of erythrocyte MDA products was found in obese diabetics and obese healthy subjects compared to control healthy subjects. The changes in erythrocyte MDA concentration after H_2O_2 - induced oxidative stress, which are also documented in Figure 5, indicate that the cells of obese diabetics are the most susceptible ones to oxidative stress. The activity of xanthine oxidase was significantly increased in obese subjects but in obese NIDDM it was almost twice as of the control (Figure 6). The level of plasma MDA positivelly correlated with FIRI, indicating that increased oxidative stress may contribute to increased insulin resistance (Figure 7).

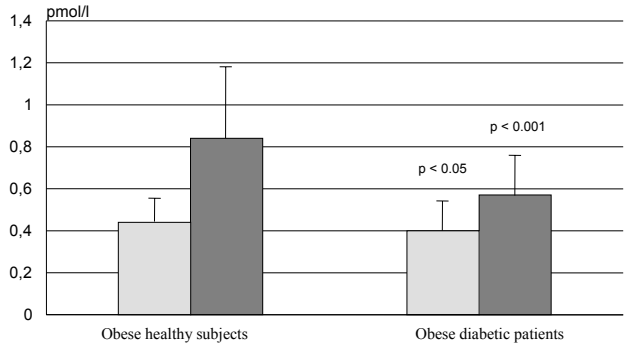

Fig. 1 – Fasting and glucagon-stimulated C-peptide in obese healthy subjects and diabetic patients

Obese subjects and obese diabetics received 1mg *iv* glucagon and blood samples were collected at basal state (0 min) and 6 min after. C-peptide was measured in both samples

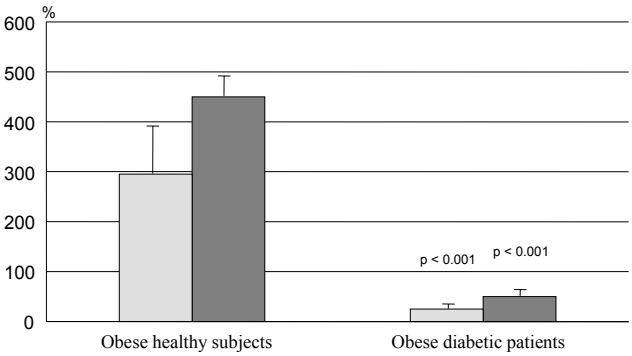

Fig. 2 – Beta-cell functional activity after glucagon load (percent of basal value)

Residual β-cell function was assessed by using the intravenous glucagon stimulation test. The values for the residual β-cell function were assessed from the insulin (light box) and glucose (dark box) concentrations by mathematical formula [21]

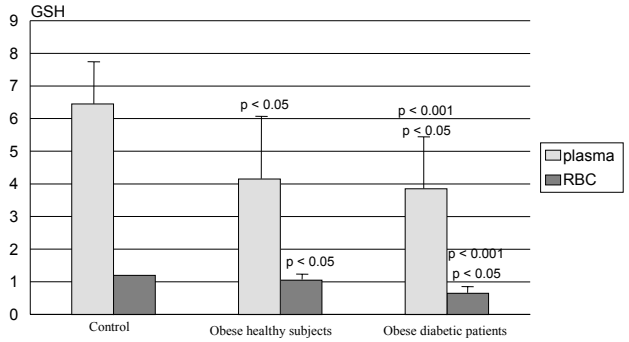

Fig. 3 – Plasma and erythrocyte (RBC) GSH in investigated groups

Reduced glutathione level (GSH) in plasma was measured by spectrophotometric assay using DTNB [23]. The level of plasma GSH was expressed as μmol/l. The level of RBC GSH was expressed as μmol/ml RBC

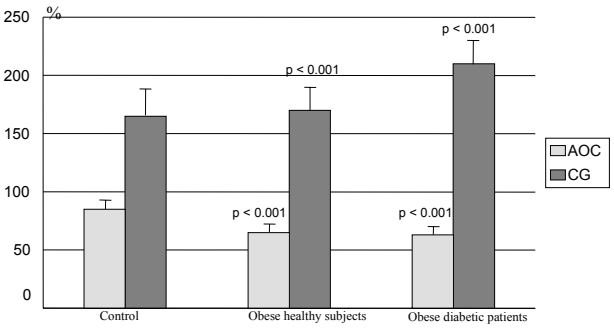

Fig. 4 – Plasma total antioxidative capacity (AOC) and plasma carbonyl group (CG) content of investigated groups (percent of basal value)

Plasma antioxidative capacity was tested by the modification of procedure of Asakawa and Matsushita [24] using an *in vitro* model of Fe^{2+}-induced peroxidation of liposome suspension (from egg yolks). Protein carbonyls was measured in plasma proteins according to the method explained in our previous report [27]

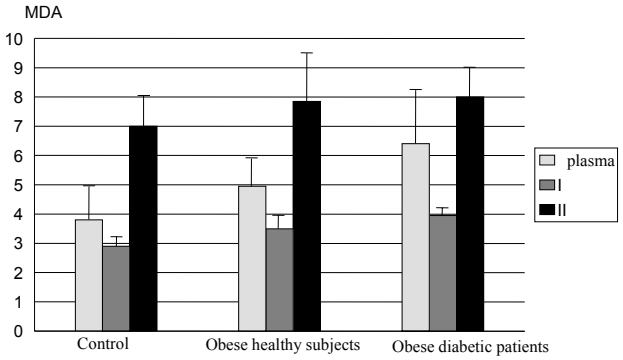

Fig. 5 – Plasma and erythrocyte (RBC) MDA at basal state (I) and after H_2O_2-induced oxidative stress (II)

Plasma lipid peroxidation was determined by thiobarbituric acid (TBA) reactivity [25]. The level of MDA was expressed as μmol/l. The level of lipid peroxidation products in erythrocytes was estimated by measuring thyobarbituric acid (TBA) reactivity [26]. For the estimation of erythrocyte susceptibility to H_2O_2-induced oxidative stress, the peroxidative challenge was induced by addition of an equal volume (1.0 ml) of H_2O_2 in isotonic saline-azide solution (final H_2O_2 concentration 3.0 mM). Results were expressed as nmol MDA/ml RBC

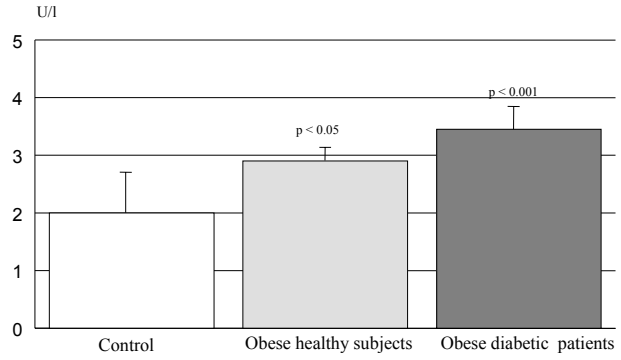

Fig. 6 – Xanthine oxidase activity in plasma of investigated subjects

The activity of xanthine oxidase (U/l) was measured according to the measurement of uric acid liberation

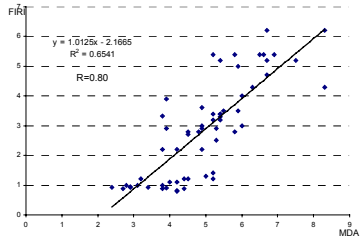

Fig. 7 – Correlation between MDA and FIRI

The coefficient of the correlation (R) between malondialdehyde (MDA) and index of fasting insulin resistence (FIRI) was obtained by linear regression analysis and by regression equation

Discussion

The accumulation of fat and BMI closely correlates with the markers of systemic oxidative stress [2, 29, 30]. It was documented that the adipose tissue represents a major source of the elevated plasma free radicals in obesity, because of the increased NADPH oxidase activity and elevated level of fatty acids. A high level of mRNA expression of the transcription factor PU.1, which upregulates the transcription of the NADPH oxidase gene in adipose tissue of obese mice was documented [30]. Byproducts of lipid peroxidation, such as *trans*-4-hydroxy-2-nonenal and malondialdehyde, are documented as potent chemoattractants, which may cause the infiltration of macrophages and inflammation in adipose tissue during obesity [2, 31–33]. They may be the important source of inflammatory cytokines, are also known to produce free radicals via NADPH oxidase [33, 34]. In the present study, it was documented that obesity may serve as an independent risk factor for the decreased activities of antioxidants and for the associated susceptibility to systemic oxidative stress. The generation of reactive oxygen species has been suggested to occur *via* xanthine oxidase activity as well.

Plasma and cellular level of antioxidant defense system represents an antioxidant endogenous potential that can protect cells from the toxic effects of free radicals. Fall in antioxidant plasma and cellular defense (plasma antioxidative capacity, plasma GSH as well as RBC GSH) was the most obvious in obese diabetics (Figures 3 and 4). A possible reason for the decreased level in obese subjects and obese diabetics may be related to decreased glutathione reductase activity, decreased GSH synthesis by glutathione-synthetase, γ-glutamyl-cysteine synthetase, glutathione-S-transferase and the impaired transport of thiol compounds in erythrocytes [35–39]. The exogenous GSH can potentiate and modulate glucose-induced insulin secretion from rat pancreatic islets in a dose-related manner. Reduced plasma antioxidative activity which was found in obese subjects and especially obese diabetics, could be also due to the structural modifications, such as glycosylation, of antioxidative enzymes [40].

Recent results have indicated that a local increase in oxidative stress in accumulated fat causes dysregulated production of adipocytokines. It was also shown that nuclear translocation of PPARγ was inhibited in conditions of increased generation of free radicals [41, 42]. Therefore, down-regulation of adiponectin expression may be partially attributed to the decreased gene expression and smaller amount of nuclear PPARγ under increased oxidative stress. Oxidative stress is known to impair both insulin secretion by pancreatic β cells [13] and glucose transport in muscle and adipose tissue [11, 12]. The reactive oxygen species (ROS), such as H_2O_2, are produced transiently in the response to insulin stimulation and also act as a second messenger for insulin signaling in adipocytes via NADPH oxidase [43–45]. A transient increase of intracellular ROS is important for the insulin signaling pathway, while excessive and long term exposure to ROS reduces insulin sensitivity and impairs glucose and lipid metabolism. An increased oxidative stress is capable of decreasing insulin sensitivity and peripheral insulin effectivity. Insulin is effective through the increased nitric oxide (NO) production, but in the presence of free oxygen radicals the activating system NO-guanylate cyclase-c-GMP may have impaired functional activity [46]. The results which are summarized in Figure 7 indicate that the increased oxidative stress positively correlated with FIRI. The erythrocytes of obese diabetics are more susceptible to lipid peroxidation with a free radical initiator than the erythrocytes of obese healthy subjects or the controls (Figure 5). Newly diagnosed NIDDM patients showed the more pronounced increase of circulating level of MDA in plasma and erythrocytes compared with obese healthy subjects or the control subjects. The results of the San Antonio Heart Study [47] demonstrated that hyperglycaemia itself can cause the increased oxidative stress and susceptibility to oxidation of macromolecules, including plasma lipids and proteins. Abnormalities in membrane function, including the decrease of insulin receptors and glucose transport have been already shown to exist in experimental and human obesity and diabetes, but together with the enhanced production of free oxygen radicals, they are able to damage cell membrane systems leading to the mechanical disruption and increased permeability [37, 48]. Enhanced production of free oxygen radicals could cause the damage of cells leading to the decreased secretory potential of islet cells, because they have very low free radical scavenging enzyme activities [48]. Xanthine oxidase (XO) reaction is the key catabolic reaction of purine

metabolism, representing at the same time a central mechanism of oxidative tissue injury through the production of superoxide anion radical. Its activity is significantly increased in obese healthy subjects and obese NIDDM patients (Figure 6).

Our previous results indicated that more aggressive insulin-sensitizing therapy is the therapy of choice in reducing oxidative stress in non-insulin-dependent diabetes [49]. The results of the present study may confirm current suggestions that insulin-sensitizing therapy, given together with antioxidants, may delay the onset and/or development of non-insulin dependent diabetes during obesity [50].

Conclusion

The disbalance between free radical-induced increase in lipid and protein oxidative modification, together with the decreased antioxidant plasma and cellular defences, may aggravate insulin resistance and decrease insulin-secretory response in obese subjects.

R E F E R E N C E S

1. *Brownlee M*. Biochemistry and molecular cell biology of diabetic complications. Nature 2001; 414(6865): 813–20.
2. *Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y,* et al. Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest 2004; 114(12): 1752–61.
3. *Matsuzawa Y, Funahashi T, Nakamura T*. Molecular mechanism of metabolic syndrome X: contribution of adipocytokines adipocyte-derived bioactive substances. Ann N Y Acad Sci 1999; 892: 146–54.
4. *Spiegelman BM, Flier JS*. Obesity and the regulation of energy balance. Cell 2001; 104(4): 531–43.
5. *Kahn BB, Flier JS*. Obesity and insulin resistance. J Clin Invest 2000; 106(4): 473–81.
6. *Saltiel AR, Kahn CR*. Insulin signalling and the regulation of glucose and lipid metabolism. Nature 2001; 414(6865): 799–806.
7. *Shimomura I, Funahashi T, Takahashi M, Maeda K, Kotani K, Nakamura T,* et al. Enhanced expression of PAI-1 in visceral fat: possible contributor to vascular disease in obesity. Nat Med 1996; 2(7): 800–3.
8. *Hotamisligil GS, Shargill NS, Spiegelman BM*. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 1993; 259(5091): 87–91.
9. *Fruebis J, Tsao TS, Javorschi S, Ebbets-Reed D, Erickson MR, Yen FT,* et al. Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. Proc Natl Acad Sci U S A 2001; 98(4): 2005–10.
10. *Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K,* et al. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat Med 2001; 7(8): 941–6.
11. *Maddux BA, See W, Lawrence JC Jr, Goldfine AL, Goldfine ID, Evans JL*. Protection against oxidative stress-induced insulin resistance in rat L6 muscle cells by mircomolar concentrations of alpha-lipoic acid. Diabetes 2001; 50(2): 404–10.
12. *Rudich A, Tirosh A, Potashnik R, Hemi R, Kanety H, Bashan N*. Prolonged oxidative stress impairs insulin-induced GLUT4 translocation in 3T3-L1 adipocytes. Diabetes 1998; 47(10): 1562–9.
13. *Matsuoka T, Kajimoto Y, Watada H, Kaneto H, Kishimoto M, Umayahara Y,* et al. Glycation-dependent, reactive oxygen species-mediated suppression of the insulin gene promoter activity in HIT cells. J Clin Invest 1997; 99(1): 144–50.
14. *Ohara Y, Peterson TE, Harrison DG*. Hypercholesterolemia increases endothelial superoxide anion production. J Clin Invest 1993; 91(6): 2546–51.
15. *DeFronzo RA, Ferrannini E*. Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. Diabetes Care 1991; 14(3): 173–94.
16. *Yki-Jarvinen H*. Pathogenesis of non-insulin-dependent diabetes mellitus. Lancet 1994; 343(8889): 91–5.
17. *Yki-Jarvinen H*. Role of insulin resistance in the pathogenesis of NIDDM. Diabetologia 1995; 38(12): 1378–88.
18. *Boden G*. Fatty acids and insulin resistance. Diabetes Care 1996; 19(4): 394–5.
19. *Ceriello A, Pirisi M*. Is oxidative stress the missing link between insulin resistance and atherosclerosis? Diabetologia 1995; 38(12): 1484–5.
20. *Paolisso G, Giugliano D*. Oxidative stress and insulin action: is there a relationship? Diabetologia 1996; 39(3): 357–63.
21. *Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC*. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28(7): 412–9.
22. *Duncan MH, Singh BM, Wise PH, Carter G, Alaghband-Zadeh J*. A simple measure of insulin resistance. Lancet 1995; 346(8967): 120–1.
23. *Beutler E, Duron O, Kelly BM*. Improved method for the determination of blood glutathione. J Lab Clin Med 1963; 61: 882–8.
24. *Asakawa T, Matsushita S*. Thiobarbituric acid test for detecting lipid peroxides. Lipids 1980; 14: 401–6.
25. *Andreeva LI, Kozhemiakin LA, Kishkun AA*. Modification of the method of determining lipid peroxidation in a test using thiobarbituric acid. Lab Delo 1988; (11): 41–3. (Russian)
26. *Jain SK, Levine SN, Duett J, Hollier B*. Elevated lipid peroxidation levels in red blood cells of streptozotocin-treated diabetic rats. Metabolism 1990; 39(9): 971–5.
27. *Kocić G, Pavlović D, Pavlović R, Nikolić G, Cvetković T, Stojanović I,* et al. Sodium nitroprusside and peroxynitrite effect on hepatic DNases: an in vitro and in vivo study. Comp Hepatol 2004; 3(1): 6.
28. *Baker JR, Zyzak DV, Thorpe SR, Baynes JW*. Mechanism of fructosamine assay: evidence against role of superoxide as intermediate in nitroblue tetrazolium reduction. Clin Chem 1993; 39(12): 2460–5.
29. *Keaney JF Jr, Larson MG, Vasan RS, Wilson PW, Lipinska I, Corey D,* et al. Obesity and systemic oxidative stress: clinical correlates of oxidative stress in the Framingham Study. Arterioscler Thromb Vasc Biol 2003; 23(3): 434–9.
30. *Olusi SO*. Obesity is an independent risk factor for plasma lipid peroxidation and depletion of erythrocyte cytoprotectic enzymes in humans. Int J Obes Relat Metab Disord 2002; 26(9): 1159–64.
31. *Roskams T, Yang SQ, Koteish A, Durnez A, DeVos R, Huang X,* et al. Oxidative stress and oval cell accumulation in mice and humans with alcoholic and nonalcoholic fatty liver disease. Am J Pathol 2003; 163(4): 1301–11.
32. *Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr*. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 2003; 112(12): 1796–808.

33. *Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ,* et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Invest 2003; 112(12): 1821–30.
34. *Curzio M, Esterbauer H, Poli G, Biasi F, Cecchini G, Di Mauro C,* et al. Possible role of aldehydic lipid peroxidation products as chemoattractants. Int J Tissue React 1987; 9(4): 295–306.
35. *Costagliola C, Libondi T, Menzione M, Rinaldi E, Auricchio G*. Vitamin E and red blood cell glutathione. Metabolism 1985; 34(8): 712–4.
36. *Murakami K, Kondo T, Ohtsuka Y, Fujiwara Y, Shimada M, Kawakami Y*. Impairment of glutathione metabolism in erythrocytes from patients with diabetes mellitus. Metabolism 1989; 38(8): 753–8.
37. *Yoshida K, Hirokawa J, Tagami S, Kawakami Y, Urata Y, Kondo T*. Weakened cellular scavenging activity against oxidative stress in diabetes mellitus: regulation of glutathione synthesis and efflux. Diabetologia 1995; 38(2): 201–10.
38. *Toborek M, Hennig B*. Fatty acid-mediated effects on the glutathione redox cycle in cultured endothelial cells. Am J Clin Nutr 1994; 59(1): 60–5.
39. *Hiramatsu K, Arimori S*. Increased superoxide production by mononuclear cells of patients with hypertriglyceridemia and diabetes. Diabetes 1988; 37(6): 8–7.
40. *Ookawara T, Kawamura N, Kitagawa Y, Taniguchi N*. Site-specific and random fragmentation of Cu,Zn-superoxide dismutase by glycation reaction. Implication of reactive oxygen species. J Biol Chem 1992; 267(26): 18505–10.
41. *Iwaki M, Matsuda M, Maeda N, Funahashi T, Matsuzawa Y, Makishima M,* et al. Induction of adiponectin, a fat-derived antidiabetic and antiatherogenic factor, by nuclear receptors. Diabetes 2003; 52(7): 1655–63.
42. *Shibuya A, Wada K, Nakajima A, Saeki M, Katayama K, Mayumi T,* et al. Nitration of PPARgamma inhibits ligand-dependent translocation into the nucleus in a macrophage-like cell line, RAW 264. FEBS Lett 2002; 525(1–3): 43–7.
43. *Krieger-Brauer HI, Kather H*. Human fat cells possess a plasma membrane-bound H2O2-generating system that is activated by insulin via a mechanism bypassing the receptor kinase. J Clin Invest 1992; 89(3): 1006–13.
44. *Krieger-Brauer HI, Kather H*. Antagonistic effects of different members of the fibroblast and platelet-derived growth factor families on adipose conversion and NADPH-dependent H2O2 generation in 3T3 L1-cells. Biochem J 1995; 307(Pt 2): 549–56.
45. *Baynes JW*. Role of oxidative stress in development of complications in diabetes. Diabetes 1991; 40(4): 405–12.
46. *Wittmann I, Nagy J*. Are insulin resistance and atherosclerosis the consequences of oxidative stress? Diabetologia 1996; 39(8): 1002–3.
47. *Chang KJ, Huang D, Cuatrecasas P*. The defect in insulin receptors in obese-hyperglycemic mice: a probable accompaniment of more generalized alterations in membrane glycoproteins. Biochem Biophys Res Commun 1975; 64(2): 566–73.
48. *Cuendet GS, Loten EG, Jeanrenaud B, Renold AE*. Decreased basal, noninsulin-stimulated glucose uptake and metabolism by skeletal soleus muscle isolated from obese-hyperglycemic (ob/ob) mice. J Clin Invest 1976; 58(5): 1078–88.
49. *Pavlović D, Kocić R, Kocić G, Jevtović T, Radenković S, Mikić D,* et al. Effect of four-week metformin treatment on plasma and erythrocyte antioxidative defense enzymes in newly diagnosed obese patients with type 2 diabetes. Diabetes Obes Metab 2000; 2(4): 251–6.
50. *Chiasson JL*. Pharmacological and surgical intervention for the prevention of diabetes. Nestle Nutr Workshop Ser Clin Perform Programme 2006; 11: 31–9.

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